Effects of Predictability of Feeding Routines on the Behaviour and Welfare of Captive Primates

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Abstract

The effects of variations in the predictability of appetitive events, such as feeding, have rarely been studied in animals in general or primates in particular. Feeding animals on highly predictable temporal schedules often results in the performance of food-anticipatory activity (FAA), characterised by increased arousal and activity and thought to be detrimental to welfare. Temporally unpredictable feeding schedules have been interpreted as resulting in improved welfare. However, if feeding is made unpredictable by preceding it with an unreliable signal, it may result in frustration and aggression. It is suggested here that two distinct but overlapping types of predictability exist. 'Temporal' predictability describes whether an event occurs at fixed or variable intervals, whereas 'signalled' predictability relates to the reliability of a signal preceding the event. This thesis examines the effects of each of these types of predictability in relation to feeding.

Welfare was assessed in laboratory-housed common marmosets *(Callithrix jacchus)* using behavioural measures, which were identified in the context of the routine stressor of human handling and weighing. The signalled and temporal predictability of presentation of a desirable titbit was subsequently experimentally manipulated. It was found that temporally unpredictable presentation of food, preceded by an unreliable signal, was associated with substantially increased stress-related behaviours in this species. If no signal was used, stress increased to a moderate level, but if the food delivery followed a reliable signal there were few behavioural changes compared to control animals. Temporally predictable feeding, without a signal, was associated with lower rates of stress-related behaviour than temporally unpredictable, unsignalled feeding. However, deviations from this temporally predictable schedule, representing delays to feeding, resulted in marked increases in stress. The results were confirmed with a further study, worked around existing feeding routines and using a different primate species, the stump-tailed macaque *(Macaca arctoides)*. Based on these findings it is suggested that the most beneficial schedule for feeding captive primates is a temporally unpredictable one, which appears to buffer animals against the negative effects of delays as well as minimising FAA. Presentation of a reliable signal before food delivery appears to minimise the stress

intrinsically associated with a temporally unpredictable routine. These recommendations represent a simple and inexpensive method of improving the welfare of captive primates.

Chapter 1

The concept of animal welfare

1.1 The definition of animal welfare

The subject of animal welfare tends to generate intense and emotive argument, and is currently of great public concern (Barnard & Hurst, 1996). There is increasing consideration in many countries of the welfare of animals used in scientific research, teaching, recreation and as a source of food (Appleby, 1999; Moberg, 1986). The field of animal welfare has become a focus of scientific study as society has increasingly looked to scientists for guidance regarding its treatment of animals (Brambell, 1965; Fraser *et al*, 1997; Thorpe, 1969). In order to discuss and assess welfare, scientists must, at least implicitly, begin with a definition of the concept (Appleby & Sandøe, 2002). However, the concept of animal welfare is a broad one, and without a simple, authoritative definition, as individual perceptions differ on the subject. A combination of philosophical controversies concerning the definition of animal welfare and scientific problems associated with its assessment result in a field that often seems confused and contradictory (Mason & Mendl, 1993).

Duncan and Fraser (1997) propose a logical framework for the conceptualisation of animal welfare in an attempt to make sense of this philosophical and methodological minefield. They suggest that welfare depends on a combination of the following three components, none of which is adequate when considered alone:

1. Welfare is dependent on what animals feel
2. Satisfactory functioning of the animal's biological systems is paramount
3. Animals should be allowed to live natural lives

Each of these elements will now be considered separately.

1. Welfare and animal feelings

The ability of animals to experience subjective feelings is central to the animal welfare debate. Historically, animals were viewed as unconscious automata, devoid of feelings and awareness. This was initially largely due to the work of the 17th century French philosopher Descartes. In his view, only humans were capable of subjective awareness, which he considered a gift from God. He claimed, in his Discourse on Method, published in 1637, that not only

> 'do the beasts have less reason than men, but they have no reason at all' (Descartes, 1637, p. 45).

Animals, according to Descartes, consist of mechanical systems with no associated mind. Without mind, they are incapable of having any mental attributes such as thought, perception, or pain. However, opposition to this notion was expressed as long ago as the eighteenth century, by the philosopher Bentham (1789). He considered that animals could feel pain regardless of whether they possessed the ability to reason.

> 'The question is not, Can they reason? Nor, Can they talk? But, Can they suffer?' (p. 283).

Today, public opinion is changing, and as animal suffering becomes more of a cause for concern, welfare is increasingly seen in terms of animal subjective experience (Curtis & Stricklin, 1991; Rollin, 1996). Without the existence of feelings, welfare would be irrelevant, and all that would be important would be the effective and productive running of the animal, similar to the maintenance of a machine. Accordingly, the term welfare cannot be applied sensibly to plants (Duncan, 1993). Philosophers (*e.g.* Midgely, 1983; Rollin, 1992; Singer, 1990) and animal welfare scientists (*e.g.* Dawkins, 1988; Duncan, 1996) writing within the past two decades agree that the capacity of animals to experience suffering is fundamental to the welfare debate.

> 'To be concerned about animal welfare is to be concerned with the subjective feelings of animals, particularly the unpleasant subjective feelings of suffering and pain' (Dawkins, 1988, p. 209).

Descartes introduced the idea of dualism, in which the body and mind were seen as separate entities, the mind being a rather mysterious, spiritual construct, private and inaccessible. This legacy lives on, and although subjective feelings are no longer seen as heaven-sent, they are still considered to be private and inaccessible to external observations. The nature of science as it is today means that it is seen as unacceptable to study anything that cannot be directly observed and quantified. The majority of scientists, therefore, consider feelings, which are of an inner, subjective nature, impossible to study in an objective and scientific way.

A variety of objective but indirect measures, based on health, longevity, reproductive success, and disturbances to behaviour and physiology, are consequently used in an attempt to infer what an animal is feeling (Duncan & Fraser, 1997). However, there is no purely objective way of combining such variables to give an overall measure of the way the animal is feeling, and therefore its welfare (Fraser, 1995). When using indirect methods to evaluate welfare, various logical steps and assumptions must be made, which are all open to criticism (Duncan & Fraser, 1997). The assessment of welfare also inherently involves value judgements about what is better or worse for animals (Fraser, 1995; Fraser *et al*, 1997; Tannenbaum, 1991), and cannot be considered a purely empirical subject. Many scientists therefore prefer to base their investigations on a more concrete concept of welfare based on the health and normal functioning of animals.

2. *Welfare and the normal functioning of the animal body*

Scientists following this approach believe that welfare is related to the normal functioning of an animal's biological systems. It is generally agreed that animals suffering debilitating diseases, injury and malnutrition, or kept in conditions that result in the development of physical deformities, are not experiencing good welfare (Dawkins, 1998; Fraser, 1995; Wolfensohn & Lloyd, 1994). Positive welfare, by contrast, is related to genetic fitness (Fraser & Broom, 1990) and is indicated by good

growth, reproduction, longevity and normal biological functioning (Duncan & Fraser, 1997). This approach is intuitively appealing, as changes in biological functioning are easier to detect than changes in subjective experience. For example, it is easier to detect when an animal is injured or malnourished than when it is in pain or hungry (Duncan & Fraser, 1997).

However, researchers disagree as to exactly how biological functioning and subjective feelings relate to an animal's quality of life. For example, McGlone (1993) states that an animal does not experience poor welfare just because it 'feels poorly' (p. 28). Welfare is only negatively affected when damage to physiological systems is such that survival or reproduction are negatively affected. Many veterinarians and others with practical responsibility for animal care believe that,

> 'taking care of an animal's physical health will automatically take care of its mental health' (Hughes & Curtis, 1997, p. 110).

Taylor (1972), following this approach, believes that intensively farmed animals experience better welfare than their extensively farmed counterparts. He believes that the shelter, nutrition and care provided for animals housed in intensive systems more than compensate for keeping them in such unnatural conditions.

Broom (1991) states that health problems such as disease or injury automatically result in reduced welfare, whether or not the animal is aware of the problem. For example, when an injured or diseased animal is asleep or anaesthetised, during which the unpleasant subjective experience associated with its condition ceases, its welfare is still poor. Clinical signs of disease may therefore be used as indicators of welfare.

In contrast, Hughes and Curtis (1997) believe that ill health may have an adverse effect on welfare, although the welfare implications of ill health caused by disorders such as disease, parasitism, and injury will depend on the extent to which they are associated with pain, distress or discomfort. The amount of suffering associated with various different disorders differs greatly, and therefore so does their effect on welfare. The amount of pain or distress suffered by the animal cannot only be assessed from

pathological changes. Gonyou (1993) states that, although welfare is dependent on animal feelings, it is impossible to assess these scientifically. Biological, physiological and pathological measures are therefore useful as convenient and adequate, if indirect, welfare indicators.

Such discrepancies between the functioning of an animal and its welfare represent a problem with this approach for the assessment of welfare. Problems may also arise if different physiological measures lead to different conclusions, as there are significant problems in combining all the different possible types of physiological measures into a single measure of welfare (Mendl & Deag, 1995). The responses of an animal to environmental challenge, such as cold or lack of food, may be viewed as a continuum, ranging from normal, minor adjustments to significant disturbance or abnormality (Fraser *et al*, 1997). Welfare scientists must decide at what point along such a continuum the welfare of the animal may be said to be adversely affected (Barnett & Hemsworth, 1990; Mendl, 1991). For example, Barnett and Hemsworth (1990) suggest that an increase in free corticosteroids of more than 40% may indicate a risk to welfare. However, Mendl (1991) warns against dogmatic adherence to such a cut-off point, which may be considered to be arbitrary, and could result in welfare problems being overlooked in animals that fall below it.

3. Welfare and the natural lives of animals

The third view considers that, in order to optimise their welfare, animals should be kept in natural environments and allowed to develop and use their natural behavioural adaptations. Kiley-Worthington (1989) states that an optimal environment is

> 'one in which the animal is able to perform all the behaviors in his repertoire.' (p. 333).

Scientists using this conception of welfare use the behaviour of the wild animal as a standard against which to assess the welfare of captive animals of the same species (Chamove & Anderson, 1989; Hediger, 1969; Lindburg, 1988; Veasey *et al*, 1996).

Differences in time-budgets are thought to indicate problems with the captive environment, which result in reduced welfare.

However, this approach is not based on a scientific foundation; rather it may have developed purely as a consequence of intuitive perception (Hughes & Duncan, 1988; Veasey *et al*, 1996). There has been much controversy in recent years as to whether animals actually need to perform certain behaviours, and whether their welfare is impaired if such behaviours are prevented. The concept of 'behavioural needs' first came to prominence with the publication of the Brambell Report (1965). It was suggested that environmental restriction of normal behaviour could prove harmful to an animal's welfare. The report was largely based on the then popular 'psychohydraulic' model of behaviour devised by Lorenz (1950).

Lorenz' model envisaged the tendency, or motivation, to perform particular behaviour patterns as building up with time within the animal, as a result of accumulation of 'action-specific energy'. He analogised this as water building up inside a reservoir. Performance of the appropriate behaviour would empty the tank, and cause a reduction in this tendency. At normal levels of motivation the behaviour would only be elicited by appropriate stimuli. However, in the absence of these stimuli and the subsequent non-performance of the behaviour, energy would build up in the 'reservoir', until the behaviour was triggered by irrelevant stimuli, or even in the apparent absence of stimuli ('vacuum behaviour', *e.g.* sham dustbathing in the domestic fowl, *Gallus gallus domesticus*).

The model suggests a necessity for animals to perform natural patterns of behaviour. This has implications for housing systems, in that the environment must allow a normal repertoire of behaviour to be expressed. This formed the basis for the Brambell Report (1965), and other legislation since (*e.g.* MAFF, 1983).

Lorenz' model attempts to give a single broad explanation for many different types of behaviour. It is useful for considering certain behaviours, such as eating, drinking and even dustbathing in domestic fowl (Vestergaard, 1980).

However, it is not able to explain other behaviours, such as aggression, which do not appear to build up over time. The model has been heavily criticised, for reasons such as its lack of a solid scientific foundation and its dissimilarity to what actually goes on in the real animal, and is now largely discredited in its original form (Dawkins, 1995).

Baxter (1988) believes that Lorenz' model, in as much as it implies that the behavioural needs of an animal are exactly equal to the full repertoire of its natural behaviour, has made it difficult to differentiate the needs of animals from their preferences, and even their whims. Baxter (1983) postulated a model that opposes the idea of behavioural needs, suggesting that behaviour *per se* is unimportant to the animal; what matters to it are the functional consequences of the behaviour. Motivation to perform a behaviour is switched off when the external stimuli eliciting it are perceived to have changed, and returned to a desired state or set point (Baxter, 1983). It follows that if an animal can be restored to its desired set point, through the performance of the behaviour itself or by physiological or environmental manipulation, then the concept of behavioural needs will never arise.

This model again has important implications for animal husbandry, suggesting that as long as animals' functional requirements are taken care of, they should not experience reduced welfare upon being placed in a barren environment in which normal behaviours cannot be carried out. However, it fails to explain findings such as that by Hughes and co-workers (1989), in which domestic hens with access to nests, built by themselves for egg laying the previous day, still performed nesting behaviour. Hughes and co-workers (1989) interpreted this as meaning, contrary to Baxter's model, that nest-building behaviour was motivated by factors other than the functional consequences of the behaviour, at least in the case of domestic fowls.

Hughes and Duncan (1981) consider that the dichotomy between the two types of model discussed above is a false one, and they are in fact two ends of a

continuum. At one end of this continuous distribution are behaviours governed largely by internal factors, such as eating and drinking, and at the other end are those mainly triggered by external stimuli, such as predator avoidance behaviour. Between these two extremes lie behaviours where internal and external events are of equal influence. The model implies that when behaviour is seen in the absence of appropriate external stimuli, it is probably influenced by strong internal motivation, which suggests it may be a behavioural need, and of significant importance to the animal.

Hughes and Duncan (1988) allege that both the Lorenz and Baxter models are too simplistic, and attempt to reconcile aspects of both in a more complex model. They claim that the concept of behavioural needs is central to the debate on animal welfare, and should not be dismissed because the original models explaining it have been found to be inadequate. Lorenzian ideas are incorporated in to this model, in that behaviour mainly controlled by external factors will eventually rise above threshold levels. As in Baxter's (1983) model, functional consequences of the behaviour switch off motivation. However, this occurs indirectly, by action on organism variables, and not on motivation itself, as Baxter suggests.

Welfare problems will occur, according to this model, when motivation rises above threshold levels, yet the environment does not allow for naturalistic behaviour. Even if the behaviour is performed in a vacuum context, lack of appropriate functional consequences mean that motivation will not be reduced. This could explain the development of stereotypies, often seen in animals kept in barren environments – the behaviour occurs in an inappropriate context, and therefore the effects necessary to switch it off do not occur. The animal may then enter a 'closed loop' of behaviour, from which it cannot escape. Hughes and Duncan (1988) conclude that animals show evidence of behavioural needs, and these should be catered for in the design of husbandry systems.

Regardless of whether or not animals have a need to perform certain behaviours, it remains the case that some behaviours seen in wild animals are triggered by adverse circumstances, such as fleeing from a predator or huddling and fluffing up the fur in cold weather (Duncan & Fraser, 1997). Environments resulting in such behaviours are likely to increase suffering rather than reduce it (Fraser *et al*, 1997). Captive giraffe may spend more time lying down in captivity than they would in the wild (Veasey *et al*, 1996). This behaviour is rarer in the wild due to the risks of predation, and it has been argued that such changes to the behavioural repertoire seen in captivity do not necessarily indicate reduced welfare. Instead, behavioural changes seen in captivity must be associated with negative effects or conditions in order for them to be considered indicative of reduced welfare (Veasey *et al*, 1996).

There are also many practical problems with the implementation of comparisons between wild and captive behaviour as a welfare indicator (review by Veasey *et al*, 1996). For example, there may be considerable individual variation within both captive and wild populations, making interpretation of results difficult (Veasey *et al*, 1996). In common with one of the problems associated with the biological functioning approach, there may be problems in defining a cut-off point at which differences in behaviour between captive and wild animals constitutes a welfare risk (Veasey *et al*, 1996). Although the technique may be useful in combination with other measures, the sole use of a direct comparison of captive and wild behaviour has been criticised as a tool for the assessment of animal welfare (Dawkins, 1980; Hughes & Duncan, 1988; Veasey *et al*, 1996).

The three conceptualisations of animal welfare discussed often give rise to similar conclusions. This is unsurprising as, from an evolutionary perspective, forces of natural selection mean that only characters conferring a biological advantage will survive over generations (Hinde, 1975). Natural behaviour ought to be adaptive in that it should generally aid survival, health and reproduction (Duncan & Fraser, 1997).

Subjective experience should also be adaptive, in that pleasant and unpleasant feelings result in behavioural flexibility, enabling performance of actions beneficial to fitness as well as avoidance of harmful situations (Dawkins, 1980; 1998; Fraser & Duncan, 1998). Some reactions, such as pain and fear, are thought to be 'unpleasant by design' (Nesse & Williams, 1995, p. 26).

> 'Pain evolved because, by being unpleasant, it keeps us away from the larger evolutionary disaster of death.' (Dawkins, 1998, p. 308).

When natural behaviour and subjective feelings contribute to biological functioning, animal welfare assessments based on the three approaches should result in similar conclusions (Duncan & Fraser, 1997).

However, assessments of welfare based on the three different conceptions do not always lead to the same conclusions. For example, followers of a feelings-based approach might consider the welfare of a marmoset housed in a breeding group in a laboratory as poor, as it shows high levels of scent-marking, thought to indicate anxiety, and attempts to escape from its cage when the opportunity arises. A functioning-based conception, however, might lead to the conclusion that the welfare of the animal is good, as it is warm and well fed, breeding well and free from injury or disease. If a naturalistic framework is used, however, welfare might again be considered to be inadequate, as the animal is housed in unnatural conditions that prevent the performance of natural behaviour. Such disagreements arise from value-laden assumptions on the part of different observers as to what factors are important in order for animals to have a reasonable quality of life (Fraser *et al*, 1997).

Another reason for disagreement between various types of measures is that the environment in which the animal is kept does not correspond to that in which it evolved. For example, nest building has evolved naturally in sows shortly before farrowing, in order to provide piglets with a warm, protected environment, thus maximising their chances of surviving. Frustration may occur if this behaviour is prevented in captivity. This is despite the fact that keeping a sow in a small, barren (but warm and clean) stall

without access to nesting materials generally results in good health and a high rate of piglet survival. Welfare of this sow and her piglets would be considered good by a functioning-based approach, but poor by feelings or natural behaviour-based approaches. The natural behaviour (nest building) and subjective feelings (frustration, if nest building is prevented) are no longer adaptive in the captive environment (Duncan & Fraser, 1997).

When different measures of welfare result in such conflicting conclusions, many researchers (*e.g.* Broom, 1991, 1996; Dawkins, 1980, 1983, 1998; Duncan & Dawkins, 1983; Fraser & Broom, 1990) recommend that many different types of measurement should be taken, and a consensus formed from interpretation of them all. Dawkins (1983) emphasises that the use of just one measure of welfare is dangerous, and all possible measures should be used in order to gain the most accurate picture of welfare possible. A practical tool for analysis of many of the factors likely to affect the welfare of captive animals, touching on all the above areas, is that of the Farm Animal Welfare Council's Five Freedoms (Anonymous, 1992). These were primarily used as a tool of analysis of the welfare of farm animals in various husbandry systems, but may be applied to any captive animals, such as those maintained in laboratories or zoos (Webster, 1994). The five freedoms are as follows:

1. Freedom from thirst, hunger and malnutrition
2. Freedom from discomfort
3. Freedom from pain, injury and disease
4. Freedom to express natural behaviour
5. Freedom from fear and distress

The five freedoms incorporate all three of the concepts put forward by Duncan and Fraser (1997); feelings (*e.g.* hunger, discomfort), biological functioning (*e.g.* malnutrition, disease) and the expression of natural behaviour. Again it must be stressed that when evaluating welfare, these attributes should not be considered in isolation; a true impression will depend on the assessment of all of them. This may include consideration of the health and pathology, physiology and behaviour of animals.

It should, however, be borne in mind that even if a battery of measures is collected, this will never constitute hard scientific evidence that an animal is or is not suffering. Rather, it will form a collection of evidence that may be used to make inferences about welfare, which will remain ultimately subjective (Mason & Mendl, 1993).

Although assessment of animal welfare depends on a definition of the concept, it has also been argued (Appleby, 1999; Fraser, 1995) that welfare scientists spend too much time discussing the question, 'what is animal welfare?' and not enough concentrating on specific problems. Appleby claims that 'We can make progress without definitions' (1999, p.19), whereas Fraser states that

> 'Instead of trying to 'measure' animal welfare, scientists should see their task as identifying, solving and preventing animal welfare problems'. (Fraser, 1995, p. 113).

Accordingly, this review will now move on to methods used by scientists in an attempt to gain an understanding of how an animal is feeling, and therefore, its welfare.

1.2 The assessment of animal welfare

As has already been stated, the animal welfare field is concerned with the subjective experience of animals. However, traditional science involves the study of processes that may be observed directly (Duncan & Fraser, 1997). The challenge of researchers in animal welfare is to understand the feelings and emotions of animals, which,

> 'like the movement of subatomic particles, cannot be observed directly.' (Duncan & Fraser, 1997, p.23).

Some physiological and behavioural measures may nevertheless be reflective of these subjective, hard to assess, hidden states.

There have been two main methods of correlating observable changes in animal behaviour or physiology with changes in welfare (Mason & Mendl, 1993). The first is to administer stressors, consisting of stimuli that the experimenter feels sure are unpleasant to the animal, such as electric shocks or removal from the social group. Changes in

behaviour and physiology in response to this challenge are recorded, and other situations that elicit comparable responses are judged to be similarly unpleasant to the animal (Mason & Mendl, 1993). The second is to argue, by analogy, that suffering in animals results in similar behavioural and physiological changes to those seen in humans experiencing suffering (Dawkins, 1990; Sandøe & Simonsen, 1992).

The experiments described in this thesis largely utilise the first method. The study described in Chapter 4 aimed to define measures of welfare in the common marmoset *(Callithrix jacchus)*, using the administration of a mild stressor to stimulate and identify stress-related behaviours in this species. Levels of circulating cortisol, a stress hormone, were also measured in these animals. The rationale of this was that if a correlation between cortisol and a particular behaviour could be made, this would increase the validity of the behaviour as an easily used and non-invasive welfare indicator. The behaviours identified in this study were used as indicators of stress in the experiments described in Chapters 5 and 6. The experiment described in Chapter 7 used the stump-tailed macaque *(Macaca arctoides)* as a study animal. Behaviours identified by other researchers as being related to stress were used as welfare indicators in this study. Both physiological and behavioural measures of stress were therefore used in an attempt to gain an understanding of the subjective experiences of the study animals.

1.2.1 Physiological measures

The stress response may provide an objective criterion for the assessment of welfare (Moberg, 1986). Well being may be threatened if the animal is stressed, particularly if that stress is intense or of a long-term nature. Chronic stress, for example, may eventually become exhausting, impairing welfare as the animal fails to sustain fitness and suffers from an increasing sense of malaise (Webster, 1994). Situations evoking behavioural, autonomic or neuroendocrine stress responses may therefore indicate a threat to the welfare of animals.

Many different physiological measures are affected by stress and therefore may be used as welfare indicators. Measures that may be useful in the short term include heart-rate, respiratory rate and body temperature, adrenal axes (adrenaline and noradrenaline) and other hormones. Measures that are more useful in evaluating welfare over longer time periods include reproductive success, longevity, weight changes, cardiovascular and blood parameters, immune system functioning and disease incidence. Glucocorticoids (cortisol and corticosterone) may be useful in both short- and long-term studies (Broom & Johnson, 1993).

This review will now describe the theory of stress, its relationship to animal welfare, and look at the effects of psychological factors on these concepts. The physiological measure of stress adopted by the study described in Chapter 4 of this thesis was that of the glucocorticoid cortisol, and therefore of the various physiological measures available, this will be the main focus of this section of the review.

The concept of stress

Hans Selye, the originator of the stress concept, defined stress as 'the nonspecific response of the body to any demand made upon it' (Selye, 1973). He discovered that exposure to a variety of noxious stimuli, such as loud noises, adverse temperature and pathogens, led to consistent changes in the rats he tested. The changes he saw were gastro-intestinal ulcers, shrinkage of immune system tissues and secretion of glucocorticoids from the adrenal cortex. Selye named this phenomenon 'stress'.

Selye believed that physiological responses to challenges (or stressors) were essentially the same, regardless of the nature (physiological or psychological) of these challenges. He claimed that these responses were due to a non-specific demand for activity, required for the performance of adaptive functions, which returned the body to homeostasis following exposure to challenges (Selye, 1973). Selye considered reaction to stressors to be triphasic (Selye, 1936), and termed this the General Adaptation Syndrome. Initially came the emergency ('alarm') reaction, which may be followed by

physiological resistance to the disturbance (Moberg, 1986). Should exposure to the stressor continue, biological adaptation may fail in the stage of exhaustion, resulting in death (Selye, 1973).

Selye's ideas were initially hugely influential in stress research, and measurement of glucocorticoids became a popular method of assessing stress, which was thought to imply reduced welfare in animals. However, the concept of the non-specific stress response has been criticised and today is largely discredited (Moberg, 1986). Mason (1971), with the benefit of improved assay techniques, showed that particular challenges had unique and characteristic effects on physiological parameters, including the entire neuroendocrine system, instead of just the adrenal cortex, upon which Selye had focussed. Mason believed these effects were context specific - the circumstances in which stressors were applied were vital to the response seen. He claimed that endocrine responses were not due to a non-specific reaction as Selye had maintained, but rather to psychological distress caused by the experimental situation itself (Mason, 1971).

Engel (1967) offers a compromise between Selye's (1973) non-specificity theory and Mason's (1971) concept of a unique response to each type of stressor. Here, two markedly different modes of response to stress are suggested. The initial, short-term alarm reaction generally leads to an active response mediated by the sympathetic adrenal-medullary system. This is the 'fight-flight' reaction, as described by Cannon (1935), in which the animal attempts to resolve the situation through its own behaviour. Depending on genetics or rearing conditions, or if the active response is futile, the long-term effect of depression of behaviour may be evoked, in which energy is conserved. This second stage is mediated by a different hormonal profile, the pituitary adrenal cortical system, and is similar to Selye's (1973) concept of stress. The effects of early experience and genetics affect how a stressor is assessed, and hence the coping patterns shown. Henry (1976) found that evidence from his own research, and from literature reviews, supported Engel's theory.

Moberg (1986) built on Engel's (1967) theory, in a model of stress in animals, which is probably the most widely accepted in the field of animal welfare today. This separates response to a challenge into three stages. Perception of a challenge to homeostasis is followed by the stress response. This varies according to factors such as experience, genotype and physiological state of the animal, and may consist of changes in behavioural, autonomic or endocrine systems. These may be costly to the animal, diverting energy away from normal biological functions. Severe or prolonged challenge may eventually lead to a 'pre-pathological' state. This may constitute a risk to the animal's mental and physical systems, which may manifest itself in various ways, such as increased susceptibility to disease, decreased reproduction or inefficient metabolism.

Psychological aspects of stress

Weiss (1972) emphasised the importance of psychological variables in the physiological manifestation of stress. He showed that the magnitude of the stress response could be strongly influenced by a sense of control over, or predictability of, the stressor. Hence, the way the animal perceived the stressor, and its available means of coping, determined the response evoked. Psychological factors can both increase and decrease arousal in response to even intense and aversive stimuli (Weinberg & Levine, 1980). The majority of research in this area has concentrated on four psychological variables (Weinberg & Levine, 1980):

1. The predictability of stressors
2. The ability of the animal to exert control over, or make coping responses during, stress
3. The effects of feedback – information available to the animal immediately following the aversive stimulus, or its response to that aversive stimulus, which indicates to it that the stimulus is over, or that it has made the correct response
4. The previous history of the animal with regard to the above factors

The review in Chapter 2 will concentrate on the behavioural and physiological effects of the predictability and controllability of potential stressors, the relationships between these two variables and the effects of feedback in the context of predictability.

The practical application of the stress concept to assessment of welfare

Increased activity in the hypothalamic-pituitary-adrenal (HPA) axis in response to physical or psychological challenge results in increases in circulating glucocorticoids. These include corticosterone in non-mammalian tetrapods, cortisol in teleost fish and cortisol, corticosterone or both in various mammalian taxa (Idler, 1972). Barnett (1987) argues that sustained elevation of glucocorticoids may provide evidence of a real or potential risk to welfare. However, measurement of short-term rise in glucocorticoids in response to transient challenge also gives valuable information about animal welfare (Broom & Johnson, 1993). Hormonal responses may also remain for some time after behavioural habituation to a stressor has occurred (Coe *et al*, 1983).

Measures of cortisol are valuable in the assessment of stress and welfare, not only because the techniques used are relatively straightforward (Terlouw *et al*, 1997), but also because cortisol has been demonstrated to show a graded response, depending on the severity of the stressor (Kvetnansky *et al*, 1984; Smith & French, 1997). There is evidence that increases in adrenocortical activity may result from exposure to short term stressors in various species of farm animal (reviewed by Barnett & Hemsworth, 1990). For example, dehorning of calves (Wohlt *et al*, 1994), castration and tail docking of sheep (Kent *et al*, 1993), restraint and isolation of castrated rams (Parrott *et al*, 1988), herding and restraint of pigs (Baldwin & Stevens, 1973; Becker *et al*, 1985) and transport of cattle and sheep (Johnston & Buckand, 1976; Purchas, 1973) all result in increases in plasma corticosteroid concentrations. In primates, levels of plasma cortisol have been shown to increase following stressors such as restraint (Goncharov *et al*, 1979; Hayashi & Moberg, 1987; Kling & Orbach, 1963a; Morrow-Tesch *et al*, 1993; Pun *et al*, 1981; Reinhardt, 1992a; review by Reinhardt *et al*, 1995a), pair formation

(Coe *et al*, 1982), high intensity noise (Hanson, 1976), novel environments (Hennessy *et al*, 1995) and maternal separation (*e.g.* Hennessy, 1986; Golub *et al*, 1981; Gunnar *et al*, 1981; Laudenslager *et al*, 1995; review by Hennessy, 1997).

Several studies have assayed urine for cortisol as a physiological indicator of stress and consequently reduced welfare (Crockett *et al*, 1993; 1994). Urinary corticosteroids have been used for many years to assess stress levels in humans (Fishman *et al*, 1962; Hamburg, 1962; Friedman *et al*, 1963; Bunney *et al*, 1965; Lundberg, 1980; reviewed by Crockett, 1998). The advantage of using urinary, faecal or salivary corticosteroids rather than those found in blood is that they may be collected without disturbing the animal unduly, whereas blood collection is more invasive, usually involving capture, restraint and possibly anaesthesia. These procedures themselves may result in elevated cortisol levels (Crockett *et al*, 1993; Reinhardt *et al*, 1995a), thus affecting experimental results. Additionally, there may be practical problems in collecting blood, especially under free or semi-free ranging conditions, or with group-housed animals (Wallner *et al*, 1999). Removal of an animal from a social group or familiar surroundings in order to carry out the procedure may also affect measures of cortisol (Hennessy *et al*, 1995; Mendoza *et al*, 1992). Sole reliance on the use of corticosteroids as measures of welfare is to be avoided, as they may be released in situations that may not represent threats to welfare, such as copulation (Szechtman *et al*, 1974) and nursing in mammals (Walker *et al*, 1992). A pragmatic approach is therefore necessary, preferably incorporating more than one method of assessment (Broom & Johnson, 1993).

It has been suggested (Wiepkema & Koolhaas, 1993) that stress and welfare are opposite to each other. However, natural conditions in which animals have evolved will evoke a certain level of arousal, and therefore it is thought that a baseline level of behavioural and physiological stress responses is normal and does not necessarily indicate reduced welfare in unacceptable conditions (Wiepkema & Koolhaas, 1993). Although some researchers state that 'stress invariably implies poor welfare' (Broom &

Johnson, 1993, p.73), a certain amount of stress may be necessary in order for the animal to maintain vigilance and avoid boredom (Wiepkema & Koolhaas, 1993).

Low-intensity arousal has been described as being 'adaptive' for animals (Dantzer & Mormede, 1983; Ewbank, 1985); Chamove and Moodie (1990) interpret this as meaning that such arousal is 'healthy'. Cotton-topped tamarins *(Saguinus oedipus)* exposed to brief threatening events showed changes in behaviour, expressing a wider range of behaviour, which was similar to that which might be expected in the wild (Chamove & Moodie, 1990). The researchers concluded that stimuli causing initial fear reactions, or other types of arousal traditionally regarded as negative, may in fact be beneficial to captive monkeys, as long as the arousal induced is brief. This further underlines the need for prudence when interpreting studies relating stress to animal welfare.

1.2.2 Behavioural measures of welfare

Of all the indicators of welfare described so far, behavioural observation is probably the most easily implemented and least invasive. Some writers (*e.g.* Poole, 1997) argue that behavioural observations are the best way of providing the researcher with information about whether an animal is 'happy or sad' (p. 116). Animals respond to their environment with different behaviours, which may in turn tell us about their needs, preferences and subjective experiences (Mench & Mason, 1997). Both 'normal' and 'abnormal' behaviour may be useful in the assessment of welfare.

The use of normal behaviour in welfare assessment

The expression of normal behaviour in animals varies greatly with species, as well as between individuals. It is therefore necessary for researchers to have a good baseline knowledge of the normal behaviour of the species in question, as well as, if possible, that of the individual animal, before attempting to infer improved or reduced welfare from observed behavioural changes (Mench & Mason, 1997). When, for

example, investigating welfare implications of a particular experimental treatment, feasible comparisons may be made between behaviours shown by the animals in question and a control group that are not subject to the manipulation. Alternatively, the experimental animal may act as its own control and post-treatment behaviours compared with baseline levels. These methods avoid the problems associated with comparing the behaviour of an animal with its wild conspecific, which were discussed earlier.

Many behaviours shown by captive animals are thought to be indicative of pain or distress. These include changes in posture, activity, gait or willingness to accept handling, as well as characteristic vocalisations (Molony & Kent, 1997). Certain behaviours, such as vocalisations, may also be increased when an animal is, for example, hungry or cold (Weary & Fraser, 1995). The frequency and intensity of such behaviours may provide researchers with information from which to infer how the animal is feeling (Mench & Mason, 1997).

The performance of displacement activities may also provide insight into an animal's emotional state (Maestripieri *et al,* 1992) and, by inference, its welfare. Displacement activities are defined as

> 'behaviour patterns (mostly body care activities) characterised by their apparent irrelevance to the situation in which they appear' (Maestripieri *et al*, 1992, p. 962).

McFarland (1993) suggests, following a review of field studies, that displacement behaviours tend to be performed in situations associated with frustration, where the ongoing or intended behaviour cannot be expressed. For example, the animal may be physically prevented from attaining its goal. Barbary doves *(Streptopelia risoria)* denied access to water by glass screens, may peck at small objects on the ground, which is thought to be displaced feeding behaviour (McFarland, 1965).

Alternatively, the displacement activity may occur in conflict situations where two incompatible behaviours are stimulated simultaneously (Maestripieri *et al*, 1992). For example, during an agonistic encounter between male chaffinches *(Fringilla*

coelebs), the two combatants may suddenly break off the encounter and preen, or peck at the ground. This behaviour seems totally irrelevant to the situation, and it has been suggested that such anomalies occur when the animal is in a state of conflict (here the conflict between the incompatible tendencies of attack and escape) and cannot decide what to do (Rowell, 1961).

Conflict situations engineered in the laboratory, such as punished drinking, result in a variety of autonomic responses such as respiratory, circulatory and thermoregulatory changes (Andrew, 1956). These physiological changes may be responsible for the fact that self-maintenance behaviours are frequently manifested as displacement activities, as they provide a set of stimuli arising in the pelage or feathers, skin and blood vessels (Maestripieri *et al*, 1992).

Webster (1994) considers that

> 'animals appear to use such displacement behaviour to reduce the intensity of acute unpleasant feelings such as frustration, anger and pain and perhaps more chronic, non-specific moods such as anxiety'. (p. 34).

Maestripieri and co-workers (1992) postulate that displacement activities in primates may be a form of coping response in stressful situations, resulting in physiological changes or redirecting the animal's attention, and thus reducing stress levels. They cite the findings of Keverne and co-workers (1989), in which allogrooming and other behaviours involving body contact were associated with the release of endogenous opioids in monkeys. It is possible that autogrooming or other displacement activities could result in a similar effect. The performance of displacement activities is affected by certain anxiogenic and anxiolytic drugs, which further supports the argument that they are associated with anxiety states (Maestripieri *et al*, 1992).

As displacement activities are normal behavioural patterns performed in an apparently irrelevant context, their identification must be contextual (Maestripieri *et al*, 1992). Displacement activities seen in primates include scratching, autogrooming, yawning and body shaking (reviewed by Maestripieri *et al*, 1992). Increased

frequencies of these behaviours may be an indication that the performer is experiencing uncertainty or anxiety.

A fundamentally different approach to the investigation of subjective experience and the assessment of welfare has been proposed by Wemelsfelder (1997). She proposes that if we change the way we look at animals and treat them as subjects for investigation in their own right, rather than as objects, a new line of enquiry is opened up. Behaviour is not considered in isolation, rather as an expressive criterion for subjective experience (Wemelsfelder, 1997). In this qualitative, rather than quantitative, approach, behavioural observations do not concentrate on the discrete, easily categorised states of behaviour that are used in traditional ethograms, such as those of foraging, grooming or resting. Instead, they include more subtle expressions of the way the animal connects one state of behaviour with the next, and interacts with its environment in a particular 'style' of behaviour (Wemelsfelder, 1997). Behaviour is then said to become a direct expression of the subjective experience of the animal. For example, an animal may behave timidly, confidently or fearfully.

However, such study of the expressive qualities of behaviour will involve interpretation by, and therefore depend on the skills of, the observer. This will mean it is not as objective as conventional science would require. This is not to undermine the value of such work; mistakes may be made at first, but with common sense and long-term detailed study of the behaviour and dynamic interactions of the animals in question they are likely to be minimised.

Reliability of investigations into subjective experience may be examined with the use of statistical tests (Wemelsfelder, 1997). High inter- and intra-observer reliability will indicate that the interpretation of the behaviour into the styles chosen is not just a random, erratic process, but is based on a common understanding. This should also make studies repeatable, and hence counter a major criticism from conventional science. Great effort should be made to ensure an

objective approach in this area, so that it becomes seen as a valid scientific discipline.

Studies on captive animals may be more restricted than those of the animal in its indigenous habitat, as their environment does not allow for as much variety and detail in their response. Yet they are crucial, as it is often the animal's reaction to its captive environment that causes the problems that those interested in animal welfare wish to address. The observations of these animals should be in conditions that allow as much behavioural expression as possible, but obviously without fundamental change to the housing system, or other aspect of the animal's circumstances, that is being investigated. The importance of prolonged and detailed observations should not be underestimated.

Wemelsfelder (1997) claims that the use of a more subject based approach in this area will give access to a rich and complex new level of knowledge which will be invaluable in the animal welfare debate. She does not believe, however, that traditional models used by science (such as those discussed earlier in this review) are useless. They are still helpful, yet do not tell the whole story, and hence should be used alongside new ideas.

The use of abnormal behaviour in welfare assessment

Captive animals sometimes perform behaviour that appears bizarre and without obvious adaptive value. Such behaviours include stereotypies (discussed in detail later in this review), self-mutilation, excessive licking of self or surroundings, polydipsia (excessive drinking behaviour), tonic immobility, hyperactivity or persistent biting or sucking of body parts, such as tails, of other individuals (Fraser & Broom, 1990; Mench & Mason, 1997). These behaviours are usually termed 'abnormal'.

There are, however, problems with this terminology. The term 'abnormal' literally means 'deviating from what is normal or usual' (Oxford Compact English Dictionary, 1996, p. 2), a definition which does not imply a value judgement. Some

researchers (*e.g.* Meyer-Holzapfel, 1968) use the word in such a sense, meaning behaviour different from that of the free-living animal, or the captive animal kept in naturalistic conditions (Mench & Mason, 1997). However, 'abnormal' is an emotionally loaded word. As soon as a behaviour is labelled 'abnormal', it tends to be automatic to assume that the animal performing it is suffering (Dawkins, 1980). The term 'abnormal' is, accordingly, often used to mean 'pathological', meaning either occurring as a result of, or causing, damage or illness to an animal (Mench & Mason, 1997; Schmidt, 1982). This may not always be the case. For example, a pet dog raising a paw in return for a desired titbit or a hamster running in a wheel are both behaviours that are abnormal in the sense that they are do not occur in wild animals, but are unlikely to be considered 'pathological' or detrimental to welfare. However, the performance of certain types of abnormal behaviour may often be an indication of poor welfare.

One category of behaviour patterns that may feasibly be considered as an indicator of welfare is stereotypic behaviours, which are diverse in form and have been the subject of many scientific studies (Mench & Mason, 1997). A stereotypy has been defined as

> 'a behaviour pattern that is repetitive, invariant and has no obvious goal or function' (Mason, 1991, p. 1015).

Stereotypic behaviour develops in sub-optimal conditions, and may result in actual physical damage to the animal performing it. For example, sows housed in stalls may rub their mouths repetitively over the bars, resulting in tissue damage (Dawkins, 1998). The performance of stereotypic and other abnormal behaviour is likely to be a valid indicator that the animal is, or has been, in a situation that is detrimental to its welfare (review by Mason, 1991). Abnormal behaviours, such as stereotypies, often occur in environments considered to be substandard, develop from behaviours associated with frustrated motivation, and correlate with other indicators of poor welfare (Mench & Mason, 1997).

However, there are substantial problems with the unquestioning acceptance of stereotypic behaviours as indicators of poor welfare. The stereotypic behaviour performed by an individual is the product of many interacting factors, not all of them related to welfare. For example, individual differences such as age, physical fitness and levels of certain hormones may affect the levels of stereotypy shown. The type of stressor an animal is exposed to, and not just its severity, may also affect stereotypies. For example, exposing rodents to chronic mild electric shock and cold, rather than being associated with abnormal behaviour, tends to result in immobility (Archer, 1979, cited in Mench & Mason, 1997). Some abnormal behaviours also appear to be socially facilitated; for example, bank voles *(Clethrionomys glareolus)* are more likely to develop locomotor stereotypies if their neighbours perform them (Cooper & Nicol, 1994). Additionally, although stereotypies tend to develop in conditions that are associated with poor welfare, they may subsequently be performed in other circumstances, becoming independent of the stimulus that led to their development. They may therefore be an indication (or a 'scar') of previous experience rather than an indication that welfare is poor at the time of their expression (Mason, 1991).

There is some evidence that stereotypies, such as bar chewing in stalled sows, head weaving in horses, and pacing in captive polar bears may actually be beneficial to the animal. This is known as the 'coping hypothesis' (Cronin, 1985; Cronin *et al*, 1985; Dantzer & Mittleman, 1993; Rushen, 1993) and contrasts with Mason's (1991) definition which states that stereotypic behaviour has no obvious goal or function. Studies on pigs, for example, suggest that endogenous opiates may be released during stereotypy and that the behaviour that produces them may in fact be a coping response (reviewed by Mason, 1991). However, Mason (1991) also showed that a substantial number of studies do not support the coping hypothesis. Rushen (1993) concluded that whilst some stereotypies seemed to be functional in that there was evidence for a coping effect, this did not hold true for all forms of stereotypy.

The presumed lack of function of stereotypies is what distinguishes them from other repetitive activities found in the behavioural repertoire of many species, such as flapping flight and grooming behaviour (Dantzer, 1986). However, it can be difficult to classify a behaviour as being non-functional and therefore stereotypic, particularly when it is an exaggerated form of a normal behaviour. To some extent, the level at which an adaptive behaviour is seen as a stereotypy, for example in licking behaviour shown by calves housed in veal crates, will always be arbitrary.

Scientists using the performance of stereotypies as an indicator of welfare must decide at what frequency stereotypies must be performed before they may be said to denote poor welfare. Broom and Johnson (1993) have proposed such a scale. Welfare by their criteria is said to be good when stereotypies are performed only occasionally, stimulated by minor frustrations. In contrast, welfare is considered very poor if stereotypic behaviour is seen for 40% or more of the animal's active time.

These points indicate that the use of abnormal behaviour as an indicator of poor welfare is not straightforward. It has been suggested, therefore, that the performance of abnormal behaviour should be treated as a sign that further investigation is necessary, as it may be indicative of circumstances that are adverse, but may also be neutral, or even beneficial (Mench & Mason, 1997). Alternatively, measurements of abnormal behaviour may be valuable in the assessment of welfare when used sensibly in combination with other behavioural and physiological measures.

It is important to realise that different physiological and behavioural measures of welfare may not always be analogous, and may therefore lead to conflicting conclusions. There are many reasons for such conflicts, some, as already discussed, resulting from value-laden assumptions as to what is important for good animal welfare on the part of different investigators. It has been suggested that some behaviours, such as stereotypies, may function as coping responses when

an animal is faced with a stressor. An increase in the coping response, which may in itself be considered an indicator of stress, may then be associated with a decrease in a different measure of stress. For example, calves showing high rates of stereotypic behaviour also showed the lowest rates of abomasal ulceration, a physiological sign of stress (Wiepkema *et al*, 1987).

The type of stressor used may also influence the response seen. Common marmosets showed an increase in locomotion when isolated. Conversely, locomotion was not affected, but increases in agonistic behaviour were seen, when individuals were housed in an unstable peer group. Both changes were associated with an increase in plasma cortisol (Johnson *et al*, 1996). Problems may occur when attempting to decide which measures of welfare are the most important. The consensus remains, however, that a wide range of measures should be used wherever possible, with a pragmatic and sensible approach taken when interrelating and interpreting them (Broom & Johnson, 1993).

1.3 The importance of animal welfare

This review has covered some of the complicated and controversial issues involved in animal welfare definition and assessment. However, it is also relevant to ask ourselves why this matter is of importance in the first place. There are two approaches that lead us to the conclusion that adequate animal welfare is important to humans in general and scientists in particular. The first approach is an ethical one, and may be applied to all animals that are affected by humans. The second approach is pertinent to laboratory situations and argues that good animal welfare is vital in laboratory animals in order to produce valid experimental results and to avoid escalating financial costs.

1.3.1 Ethical importance of good animal welfare

Ethics has been defined as 'the philosophical study of the moral value of human conduct and of the rules and principles that ought to govern it' (Collins English Dictionary, 1994). Philosophers have contributed much to the discussion of animal welfare, and have helped to stimulate informed debate (Appleby & Sandøe, 2002). In recent years there has been much analysis of the relationship between humans and animals, particularly regarding the use of animals in academic and industrial research, in agriculture and in zoos (Moran, 1987). The field of animal welfare is founded on the assumption that humans have ethical obligations to animals (Sandøe *et al*, 1997). If we did not believe that we had a duty to care for animals, assessment and improvement of the conditions in which we keep them would be irrelevant. There are several philosophical approaches pertinent to the ethical question regarding humanity's duties to animals. Four of them are described below.

The utilitarian view takes the interests of both humans and animals into account. Moral judgements regarding the use of animals, for example in research, involve an ethical evaluation of the costs and benefits to all individuals concerned. For research to be deemed justifiable by the utilitarian principle, the potential benefit to humans (or other animals) should clearly outweigh the pain and suffering that is experienced by the experimental animals (Rollin, 1985).

The Home Office (1986) Animals (Scientific Procedures) Act, designed to safeguard, as far as possible, the welfare of animals used in scientific research in Britain, adheres to utilitarian principles in its regulation of the procedures permitted. Any pain, distress or lasting harm to the animal caused by a procedure must be justified by the likely benefit of that procedure. However, such decisions involve two types of judgement, neither of which can ever be precise. Firstly, it is virtually impossible to predict how beneficial a piece if research will be in relation to reducing human or animal suffering, protecting the environment, or increasing

knowledge in any given area. Secondly, the assessment of likely suffering to animals is bound to be even more difficult than that of actual suffering, which as we have already seen is fraught with problems. Generally, both the potential benefits of research and degrees of suffering are assigned to broad categories, which are compared in a cost-benefit analysis (Martin & Bateson, 1986). Reasoned discussion is necessary to reach balanced judgements on the keeping of laboratory animals, which requires factual information relevant to the assessment and reduction of suffering of these animals.

The rights view asserts that animals have rights as 'subjects of a life' (Regan, 1983), and these rights should not be violated for the greater good of species (of whatever kind) or ecosystems. Thus, humans are not justified in using an animal in experiments for the benefit of others, human or animal. According to the rights view, there is no justification for the use of animals in science or agriculture, or for keeping them in captivity in zoos (Regan, 1995).

The species-integrity view argues that the individual animal should not be the only focus of moral concern. This ecologically based framework considers that humans also have duties to protect species. This contrasts with the utilitarian view, which considers species irrelevant, as they are not conscious entities and therefore do not have rights apart from those of the individual animals that are members of that species. Similarly, the rights view is concerned with the moral rights of individuals and does not consider the species to have any rights (Rolston, 1989).

The agent-centred view does not focus on animals as individuals or as species, and may have little direct concern for animals at all. Rather, it considers our treatment of animals to be important in terms of what it does to us as moral agents (Sandøe *et al*, 1997). A person causing unnecessary suffering to an animal is wrong, not because suffering is being increased for little or no benefit (the utilitarian view) or because the animal has rights (the rights view). Such behaviour is wrong

according to this framework because it shows a lack of care in the individual concerned.

We inherit aspects of many such approaches from our cultural background, and will tend to draw on all of them when making judgements on ethical issues (Sandøe *et al*, 1997). Rollin (1985) offers such a hybrid position, incorporating elements from the utilitarian and the rights approaches in the question of whether animals should be used in research. He states that if research is deemed justifiable by the utilitarian principle, it should be conducted in a manner that maximises the animal's potential for living its life according to its nature, or *telos*. Certain fundamental rights should be preserved as far as possible within the research, regardless of cost considerations.

Humans are moral beings; we consider the consequences of our actions to others as well as ourselves, and do not always act selfishly (Appleby, 1999). It is thought that such moral considerations arose a long time ago in terms of our evolutionary history, and the 'others' that we consider are not just humans (Appleby, 1999; Ridley, 1996; de Waal, 1996). There is, consequently, a widespread consensus between philosophers, welfare scientists and the general public that humans have a duty towards animals and should work harder to improve their welfare (Appleby, 1999).

1.3.2 Scientific importance of good animal welfare

Welfare problems during the transport and slaughter of animals raised for food may cause economic problems as well as ethical concerns. Economic losses may result from, for example, bruising, meat quality problems and even death during transport and lairage (Grandin, 1993). Likewise, animal welfare is more than just an ethical concern for scientists working with laboratory animals. Animals suffering the behavioural and physiological consequences of stress may also increase the variability of experimental results, as the extra confounding variable of stress is introduced. This may necessitate

the use of more subjects in order to gain a statistically representative sample, which will invariably also increase the financial cost of the study.

The importance of standardised responses to treatment in experimental animals has long been recognised in laboratory science, and this led to them being kept in identical environments, no matter how uncomfortable, barren or socially disturbing they were (Chance & Russell, 1997). Growing awareness that environmental conditions affecting the behaviour of animals might also affect their physiology led Chance (1957) to the conclusion that the nature of the conditions in which laboratory animals were kept was directly related to the variability of their responses. He found that the results with the lowest variance were associated with more welfare-friendly housing and handling of experimental animals.

There are various potential sources of stress to laboratory animals. Most obvious are the scientific procedures themselves, which may cause pain, suffering, distress or lasting harm (Webster, 1994). Care is taken in Britain to minimise the effects of suffering in experimental animals, especially since the introduction of the Animals (Scientific Procedures) Act (1986), which is implemented by the Home Office. This Act stipulates rules and guidelines for the breeding, housing and husbandry of laboratory animals, and is designed to protect living vertebrates used in scientific procedures. However, it is inevitable that certain procedures, such as deliberate induction of bone fractures or burns, or infliction of disease will cause severe pain or distress.

Factors other than the experimental procedures themselves may also affect laboratory animal welfare. For example, the physical environment may present challenges such as noise, overcrowding, inexpert or unsympathetic handling by laboratory technicians, or ultrasound, which may be perceived by and therefore affect rodents, dogs and smaller primates (reviews by Clough, 1984 and Poole, 1997). Normal, healthy animal subjects (in terms of both physiology and behaviour) are necessary for the majority of laboratory experiments. The stress associated with the laboratory

environment may therefore represent an uncontrolled variable in experimental studies, potentially increasing the variance of the results and even rendering affected individuals unsuitable as study animals (Poole, 1997). It is therefore important for the validity of experimental results that factors leading to behavioural and physiological abnormalities are recognised and eliminated as far as is practicable.

The social grouping in which animals are kept has been shown to affect their physiology and behaviour. For example, social animals such as squirrel monkeys *(Saimiri sciureus)* show raised levels of corticosteroids when housed alone (Mendoza *et al*, 1992). In contrast, overcrowding has been demonstrated to affect the resistance to parasites and immune response of mice *(Mus musculus)* (Brayton & Brain, 1973; Edwards & Dean, 1977). Subordinate mice also show higher levels of stress hormones than dominants with which they are housed (Hucklebridge *et al*, 1976). However, Bohus and Koolhaas (1991) conclude, following a literature review, that immune function is only affected by social situations where the animal concerned is unable to exert a measure of control. Animals housed alone will clearly be unable to influence their situation, and similarly, those unable to escape from an aggressor or overcrowding will be unable to exert control. It has been proposed that in order to maximise welfare, laboratory animals should be housed in species-appropriate social groupings wherever possible (Poole, 1997).

Non-social environmental factors may also have an important effect on welfare. For example, bright light may be aversive to nocturnal species, and has been associated with retinal abnormalities, abortion and low growth rates in rats *(Rattus norvegicus)* and mice *(Mus musculus)* (Clough, 1984). This highlights the importance of assessment of the suitability of the captive environment on a species-specific level. Routine, unavoidable husbandry procedures may also result in a certain amount of stress. The heart rate of rhesus monkeys *(Macaca mulatta)* increased during cage cleaning, and remained elevated for around two hours afterwards (Line *et al*, 1989a). It is unlikely that husbandry routines such as cage cleaning could be eliminated in the interests of

welfare; indeed, it may not be desirable to do so, as some researchers claim that low levels of stress may be beneficial to welfare (Wiepkema & Koolhaas, 1993). However, the physiological implications of such routines should be considered, for example when scheduling experimental procedures (Poole, 1997).

Studies on farm animals have indicated that handling styles and attitudes of stockpersons may have an important effect on animal welfare. For example, pigs *(Sus scrofa)* handled in a caring and sympathetic manner show increased growth and reproductive success (Hemsworth & Barnett, 1987). The same appears to be true of laboratory animals. Laboratory rabbits *(Oryctolagus cuniculus)* restrained in the routine manner developed more atherosclerosis than those handled in a more 'friendly' way (Nerem *et al*, 1980). A positive, caring attitude from laboratory staff may therefore be valuable in reducing stress in laboratory animals, thus decreasing the variability of experimental results.

Another way in which the stress associated with handling may be reduced is by training laboratory animals to cooperate during routine procedures. The Home Office (1986) code of practice for the housing and care of animals used in scientific procedures states that

> 'The least distressing method of handling is to train the animal to co-operate in routine procedures' (Sec 3, para 50).

Rhesus monkeys trained with positive reinforcement to present an arm for injection showed less diarrhoea than those that had to be restrained for the same procedure (Reinhardt, 1990a, 1992a). Restraint is known to be highly stressful to laboratory animals (Cronin, 1985; Lawrence, 1991) and any way to avoid this procedure is likely to be beneficial to welfare and hence scientific results. Findings presented in this thesis (Chapter 4) suggest that training common marmosets to urinate on command in designated areas may reduce the stress associated with the routine procedure of handling and weighing. However, the results found here may be as much due to the effects of positive human interaction as to those of training itself (McKinley *et al*, 2002).

The behavioural and physiological stress reactions of laboratory animals may be activated by social conditions, developmental history, handling and environmental stressors in the laboratory. In addition to being ethically unacceptable, these reactions may necessitate the use of a larger number of study animals in order to ensure the validity of the results, which will increase the financial cost of the experiment. Stress reactions may even make the affected animals unsuitable for use in scientific studies. Poor welfare may lead to inaccurate, invalid experimental results. It is therefore the responsibility of scientists to ensure that the welfare of their study animals is catered for as well as possible.

1.4 Attempts to improve the welfare of captive animals

Greater awareness of the problems faced by laboratory and zoo animals has led to attempts to improve their welfare by a variety of methods. For example, zoo animals are increasingly being kept in pairs or social groups, in larger enclosures, with the widespread implementation of environmental enrichment (Young, 1998). Environmental enrichment is increasingly also being used for laboratory animals, although experimental protocol may mean they remain singly housed. Environmental enrichment has been defined as a manner of changing the environments of captive animals for the benefit of the inhabitants. Behavioural opportunities arising as a result of environmental enrichment have been described as behavioural enrichment (Shepherdson, 1993). Environmental enrichment attempts to improve welfare by making the animals' environment more interesting in a way that allows them to interact with it, using behaviours that have evolved over millions of years in their natural environment (Shepherdson, 1988). The goals of environmental enrichment have been further defined as follows (Young, 1998):

1. Increase behavioural diversity
2. Reduce the frequencies of abnormal behaviour
3. Increase in the range or number of normal (*i.e.* wild) behaviour patterns

4. Increase positive utilisation of the environment
5. Increase the ability to cope with challenges in a more normal way

(p. 19)

Research has shown that environmental enrichment may have highly beneficial effects on behaviour and physiology (Shepherdson, 1994). Laboratory research on rodents has shown those raised in enriched conditions to have changed brain morphology, make fewer errors in maze-type orientation problems, be quicker to learn operant tasks, exhibit reduced emotionality, and show qualitative and quantitative increases in exploratory behaviour (reviewed by Widman *et al*, 1992).

Primates are thought to show more variety and complexity in their social relationships and interactions than any other group of animals (Wrangham, 1983). Because of this complex social organisation, and their advanced intelligence, they are thought to be particularly adversely affected by captive environments (Dickie, 1994; Erwin & Deni, 1979; Hediger, 1969; Reinhardt & Roberts, 1997). Behavioural and physiological changes as a consequence of environmental enrichment are likely to make captive primates better able to cope with stress inducing novel and uncertain situations by responding with appropriate active behaviour (Box, 1991; Carlstead & Shepherdson, 1994; Widman *et al*, 1992). Stress inducing situations in captivity may include parturition and infant rearing, as well as stress associated with handling and the experimental procedures conducted on laboratory animals. There has been a large amount of empirical study focusing on the design, implementation and effectiveness of enrichment techniques for primates. Some of these are described below.

1.4.1 Increasing cage size

Early studies found evidence that singly housed monkeys showed more abnormal behaviour when housed in small cages (Draper & Bernstein, 1963; Paulk *et al*, 1977). These findings have not been replicated in more recent experiments (*e.g.* Bayne & McCully, 1989; Crockett *et al*, 1990, 1993, 2000; Line *et al*, 1989b).

However, captive chimpanzees moved from a small to a larger and more complex enclosure showed increases in locomotion and species-typical behaviour, which was interpreted as indicative of improved welfare (Jensvold *et al*, 2001). Levels of aggression in group-housed primates have also been found to increase as cage size is reduced (Woolverton *et al*, 1989). However, increasing the size of primates' cages may not be beneficial to their welfare unless cage complexity and usable space is also increased.

1.4.2 Increasing cage complexity and the provision of novel objects

Although the presence of other animals and the physical structure of the enclosure will to a large extent determine the complexity of the captive environment, the inclusion of manipulable objects will increase this complexity (Sambrook & Buchanan-Smith, 1997). Increased complexity is thought to expand the behavioural repertoire of captive animals (McGrew *et al*, 1986; Sambrook & Buchanan-Smith, 1996; 1997; Segal, 1989). The addition of novel objects to the environments of captive primates and other animals has become standard practice as a means of improving their welfare (*e.g.* Adams *et al*, 1992; Bayne *et al*, 1993a; Bloomsmith *et al*, 1990; Brent & Stone, 1996; Chamove & Anderson, 1989; Chmiel & Noonan, 1996; Hamilton, 1991; Line & Morgan, 1991; Pruetz & Bloomsmith, 1992; Shefferly *et al*, 1993).

However, primates tend to quickly become accustomed to the presence of an enrichment device, and their use of it rapidly declines (*e.g.* Bloomsmith *et al*, 1990; Cardinal & Kent, 1998; Perkins *et al*, 1992; Watson *et al*, 1997). Many studies have found that the amount of time spent by primates using or in contact with enrichment objects dropped to a strikingly similar ten per cent or less of daylight hours (Crockett *et al*, 1989; Line & Morgan, 1991; Maki & Bloomsmith, 1989; review by Crockett, 1998). This is likely to be due to habituation, a phenomenon in

which the response of an animal to a stimulus affects its subsequent responses to the same stimulus (Hinde, 1970).

1.4.3 Feeding enrichment

There has been much study and discussion of the benefits and best implementation of feeding enrichment (see Reinhardt & Roberts, 1997, for a review). Animals do not appear to habituate readily to foraging devices and other forms of feeding enrichment (Bayne *et al* 1991, 1992; Boccia, 1989; Markowitz & Line, 1990; Murchison and Nolte 1992; Phillippi-Falkenstein, 1993). Many species of captive animal have been observed working for food, even in the presence of freely available, identical food (*e.g.* Neuringer, 1969; Reinhardt, 1994). This has been interpreted as showing an inherent drive for food gathering and processing, a drive that is often not catered for in captivity (Reinhardt & Roberts, 1997). Laboratory primates, in particular, often receive their daily food ration already chopped in a bowl, requiring no effort or skills to find or prepare it. The provision of food puzzles, woodchip floor coverings, scattered food and whole food that requires processing before consumption are designed to address this problem.

1.4.4 Social enrichment

The addition of conspecifics to the environment of social species has been described as

> 'an obvious as well as cost-effective means of providing a dynamic form of environmental enrichment' (Baer, 1998).

The provision of a dynamic and responsive aspect, such as a social companion, to an otherwise static environment appears to sustain interest and be resistant to habituation (Ranheim & Reinhardt, 1989). Social housing may lead to an increase in activity levels and a reduction in the frequency of stereotypic and abnormal behaviours (Baer, 1998). Housing primates in groups is thought to provide them

with numerous opportunities to perform many components of their species-typical behavioural repertoire (Schapiro *et al*, 1997), such as courtship, mating, playing and grooming (Baer, 1998).

There are, however, several problems and risks involved in housing animals in social groups (Baer, 1998). Crowded conditions are thought to lead to social stress, reducing welfare and immunosuppression (Baer, 1998). Even in relatively spacious conditions, social housing may be associated with agonistic interactions leading to injury and stress, disease transmission and malnutrition of subordinate animals due to lack of access to food (Reinhardt 1990b). There are also several routine management difficulties associated with social housing of animals. It is difficult to monitor appetite, food and water consumption and urine and faeces production of individuals, and there may be difficulty in identifying and treating sick or injured animals (Baer, 1998).

1.4.5 Manipulating predictability of husbandry routines

A great deal of work has been carried out focusing on the design, implementation and effectiveness of environmental enrichment techniques in recent years, some of which have been described here. Most zoos, at least in the western world, now use environmental enrichment techniques, to a greater or lesser extent, to enhance the welfare of their animals (Young, 1998). However, the standard for laboratory animals remains barren, empty cages (Reinhardt & Reinhardt, 1999a).

There are many reasons for this, but financial considerations are paramount. For example, increasing cage size is likely to be expensive (Bowden, 1988; Line *et al*, 1989b; Crockett, 1993; Hubrecht & Mason, 1993; Crockett & Bowden, 1994; Poole & Hubrecht, 1994). Housing animals in social groups often requires bigger cages (Home Office, 1986), so cost is clearly a limiting factor here. Additionally, monitoring animals sufficiently to detect and prevent aggression may be impossible in many establishments without employing extra staff. Experimental protocol may also make social housing

impractical in some cases. The time investment required to manufacture, install and clean enrichment devices such as swings and perches, in order to increase cage complexity, may be beyond the capabilities of existing staff of some busy laboratories. Similarly, time, and therefore cost, limitations may restrict the implementation of feeding enrichment.

Manipulation of the predictability of husbandry routines may represent an opportunity to improve the welfare of captive animals. Relatively simple and inexpensive modifications to husbandry routines may be easy to incorporate into the schedules of busy staff yet could have a profound impact on the well being of animals in their care. It has long been known that variations in the predictability of a stressor may have pronounced effects on the behavioural and physiological effects of stress in rats (see Chapter 2 for a full review). It is reasonable to expect that variations in the predictability of husbandry routines thought to be aversive to animals (*e.g.* cage cleaning, Line *et al* 1989a) might have similar effects on stress indices. Similarly, variations in the predictability of positive events, of which feeding is an obvious example, might affect welfare. There has been a paucity of research in this area, and studies that have been carried out have reached conflicting conclusions (*e.g.* Bloomsmith & Lambert, 1995; Carlstead, 1986; see Chapter 2). This thesis explores the effects of variations in the predictability of feeding routines on the behaviour and welfare of captive primates.

Chapter 2

Effects of predictability on the welfare of captive animals

2.1 Introduction

The predictability of an event is known to affect an animal's response to it (Weinberg & Levine, 1980). This review will examine the behavioural and physiological effects of the predictability of aversive and appetitive stimuli, and the application of experimental findings to animal husbandry in practice. The motivational consequences of predictability, however, are thought to be closely related to those of control. An event is deemed controllable if there is a difference in the likelihood of it occurring depending on an animal's behaviour (Overmier *et al*, 1980; Sambrook & Buchanan-Smith, 1997). Some researchers (*e.g.* Mineka & Hendersen, 1985) suggest that the relationship between these two factors is so profound that a full understanding of one cannot be achieved without the other. For this reason, this review will also discuss the effects of control, as well as the relationships, and potential confound, between these two variables.

Studies investigating the effects of predictability of stimuli on animal behaviour and welfare have tended to manipulate predictability in one of two ways. The most obvious method involves manipulating the temporal characteristics of the stimulus presentation, delivering it to the animal on either a fixed-time or variable-time schedule. For example, a positive stimulus, such as food, or an aversive stimulus, such as electric shock, might be delivered to animals at random times, which are irregular and therefore unpredictable. Alternatively, the stimulus could be delivered at fixed times, which are regular and therefore predictable. The second method involves preceding the stimulus with a signal. A regular signal preceding the stimulus by the same time interval will render it predictable, irrespective of whether it occurs on a fixed or variable time schedule. Variations in the predictability of the stimulus may be achieved by manipulating the

reliability of the signal preceding it. Thus, a stimulus occurring after 50% of signals will be less predictable than one occurring after 100% of signals.

No studies have thus far specifically discriminated between these two methods of varying the predictability of a stimulus. However, in this thesis I propose that the methods relate to two different types of predictability, referred to throughout the thesis as 'temporal' and 'signalled' predictability. The following review therefore discusses studies in the light of these different types of predictability.

Captive animals are invariably held in an environment that is smaller and less complex than their natural habitat (Chamove & Anderson, 1989; Buchanan-Smith, 1997). Reduced environmental complexity is generally associated with an increase in predictability, as these two factors are inversely related. Many animals have evolved endogenous clocks enabling them to predict and exploit temporal regularities of environmental food availability (Mistleberger, 1994; Roberts, 1998). However, deviations from a regular and predictable schedule of food availability can and do occur. Environmental unpredictability has been described as

> 'a single, often acute, event such as an attack by a predator or the occurrence of a snow-storm that, for hours to days, disrupts 'normal' ongoing activities by temporally diminishing food resources and by increasing energetic demands' (Reneerkens *et al*, 2002, p. 81).

Studies have shown that unpredictable environmental conditions result in elevated concentrations of glucocorticoids in a range of vertebrates, including mammals, birds, reptiles, amphibians and fish (review by Wingfield & Ramenofsky, 1999). The long-term activation of these hormones is unlikely to be adaptive in a free-living individual, as it may result in delay of puberty, suppression of growth, metabolic exhaustion from breakdown in skeletal muscle, increased susceptibility to disease and neuron death in the hippocampus (Wingfield & Ramenofsky, 1999). However, the effects of the short-term activation of glucocorticoids may be adaptive as they trigger physiological and behavioural responses to overcome the impact of the

stressor, such as suppression of reproductive and territorial behaviour, as well as facilitation of foraging and exploratory behaviour (Wingfield & Ramenofsky, 1999). For example, elevations of corticosterone are associated with increased exploration in rats *(Rattus norvegicus)* and white-crowned sparrows *(Zonotrichia leucoprys gambelii)* (Breuner, 1998; Sandi *et al*, 1996). Spatial memory is also enhanced in rats and mountain chickadees *(Parus gambelii)* when corticosterone levels rise (Luine *et al*, 1996; Saldanha *et al*, 2000).

Temporally unpredictable feeding results in elevated levels of corticosterone in captive red knots *(Calidris canutus)*, a shorebird, possibly due to a 'perception of uncertainty' (Reneerkens *et al*, 2002, p. 86). It has been proposed that this increase in corticosteroids may promote both exploratory behaviour and enhanced memory performance, which are necessary in order to find food when supplies are unpredictably variable. These costly processes are therefore tailored to the needs of the individual (Reneerkens *et al*, 2002). The responses may only be adaptive when the animal has a degree of control over its environment and, for example, is able to increase the amount of exploration in response to an unpredictable food supply. In a barren cage in the captive situation, however, increased exploration may be impossible. The inability to respond appropriately to stimuli with such adaptive behaviours may mean that motivation to perform these behaviours may not be reduced, resulting in welfare problems (Hughes & Duncan, 1988; see also section on behavioural needs in Chapter 1 of this thesis).

Although animals have evolved to cope with environments of great temporal and physical complexity, until recently a widely held view was that predictable captive environments were preferable because they offered security and consequently reduced stress (Shepherdson, 1989). It is probable that a human preference for routine is partly responsible for this, along with several scientific studies concerning the effects of predictability of aversive stimuli.

2.2 Experimental studies on the effects of predictability of aversive events

A number of behavioural studies have used electric shocks as aversive stimuli, and rendered the signalled predictability of these events high (by preceding their presentation by a conditioned stimulus acting as a signal, *e.g.* a tone or light) or low (no signal). Rats, fish and birds offered a choice between predictable and unpredictable electric shock will generally choose shock with high signalled predictability (reviewed by Badia *et al*, 1979), whether or not they are able to escape it. Predictable shock has also been found to be less behaviourally disruptive than unpredictable shock (Davis & Levine, 1982; Seligman & Meyer, 1970). These results have been taken to suggest that an element of predictability makes shock less aversive. This effect is so pronounced that rats chose signalled rather than unsignalled shock even when the predictable shock was four to nine times longer and two to three times stronger than that in the unpredictable condition (Badia *et al*, 1973).

One study (Badia *et al*, 1975) looked at both temporal and signalled predictability of shock. It was found that temporally predictable shock (*i.e.* shock given at regular intervals) was chosen by rats over temporally unpredictable shock (*i.e.* shock given at irregular intervals, making it impossible for the animal to predict exactly when it would occur), when both conditions were unsignalled. However, when a signal was introduced into the temporally unpredictable condition, this preference was reversed (Badia *et al*, 1975). This suggests that signalled predictability may be more important, or at least more perceptible, than temporal predictability.

Studies relating predictability to physiological measures of stress, however, are less consistent in their conclusions. Unsignalled and temporally unpredictable aversive stimuli have been shown to be associated with physiological stress responses such as gastric ulcers, weight loss and increased plasma corticosterone concentrations (Gliner, 1972; Weiss, 1972; Seligman & Meyer, 1970). In contrast to these findings,

there is also a large body of experimental evidence to suggest that signalled or temporally predictable shock is more aversive for animals, according to similar physiological stress indices (reviewed by Weinberg & Levine, 1980). Hennessy and colleagues (1977) altered the temporal predictability of shock by manipulating the regularity of the intershock interval. Highly temporally predictable shock, where the temporal regularity of the schedule could be used to predict the next shock presentation, was associated with a high level of pituitary-adrenal activation. Highly temporally unpredictable shock, where the animals had no cues to enable them to predict the next shock presentation, resulted in a similarly high level of activation of plasma corticoids. Moderately temporally unpredictable shocks, delivered on a schedule with moderate variability in the inter-shock interval, however, resulted in significantly lower pituitary-adrenal activation.

Various explanations have been suggested for these observed effects, and the discrepancies between findings relating predictability to stress. These have focused on the following parameters.

1. Study length

Abbott and co-workers (1984) examined the literature on physiological responses to predictable versus unpredictable shock in an attempt to explain the conflicting results of behavioural and physiological studies on the effects of predictability. The majority of these studies had used the presence or absence of a signal to render shock predictable or unpredictable. Abbott and co-workers (1984) found that the length of the experiment varied considerably between studies. They concluded that unpredictable shock is more stressful than predictable shock in short-term studies, but less stressful in long-term studies. The researchers accounted for this with the following explanation. Animals exposed to predictable signalled shock learn to discriminate periods of danger from periods of safety, according to the presence or absence of the relevant signal. Under unpredictable conditions, however,

there is a relatively steady level of threat, which initially maintains a high state of physiological arousal. When exposure to unpredictable shock is long-term, adaptation occurs, and arousal is reduced. This adaptation would not occur when there is long-term exposure to predictable shock, as physiological arousal only occurs during brief periods of signal presence.

Thus, in short-term studies, unpredictable shock leads to high arousal and possible physiological exhaustion, whereas 'safe periods' associated with predictable shock provide relief from stress, making this condition less stressful overall. However, in long-term studies, lack of adaptation in the predictable shock condition may eventually cause exhaustion, which would not occur in animals adapted to the threat of unpredictable shock.

Arthur (1986) refutes the conclusions of Abbott and colleagues (1984), claiming that their classification of studies into short- and long-term is unsatisfactory. He claims that predictable shock is more stressful in either short- or long-term studies, and that conflicting results in previous studies are due to confounding variables. If this is true, physiological evidence indicating that predictable shock is more aversive than unpredictable shock appears to contradict the behavioural evidence, which shows that animals choose predictable over unpredictable shock, given a choice (reviewed by Badia *et al*, 1979). These behavioural studies have been taken to mean that predictable shock is less aversive than unpredictable shock.

2. Measure of stress used, and the assumption that different physiological and behavioural indices of stress are directly comparable

Different measures are used to indicate physiological stress in the various studies, and these different physiological measures may not be directly comparable. For example, it is thought that gastric ulceration, which is used as a measure of chronic stress, may give a different impression of stress severity as compared to a more acute indicator such as pituitary-adrenal activation (Weinberg & Levine, 1980).

Additionally, behavioural studies generally show that animals prefer predictable over unpredictable shock (reviewed by Badia *et al*, 1979), whereas conflicting data have been presented as to whether predictable or unpredictable shock results in greater physiological stress response (reviewed by Weinberg & Levine, 1980). Behavioural and physiological measures of stress appear not to be analogous. See Chapter 1 (page 26) for further discussion of this point.

3. Preference tests may not necessarily be good indicators of stress

Miller and co-workers (1983) trained water-deprived rats to press a 'high-aversiveness' or 'low-aversiveness' lever in response to a fixed-electrode variable-intensity tail shock, in order to obtain water. This enabled the experimenters to gauge how intense the rats perceived the shocks to be. They showed that although rats prefer predictable rather than unpredictable shock, they actually perceive the former as more intense. This finding implies that preference does not necessarily indicate the stressfulness of the various conditions. Arthur (1986) claims that it is nonsensical to conclude that predictable shock is less stressful when there is evidence (Miller *et al*, 1983) that rats experience predictable shock as more intense. It appears, therefore, that predictable shock may be more aversive (in terms of physiological stress responses) than unpredictable shock under certain experimental conditions. Pitman and co-workers (1995) showed that greater predictability of shock was associated with higher plasma corticosterone and norepinephrine levels, taken to be indicative of chronic stress, supporting the conclusions of Miller and colleagues (1983). Pitman and colleagues (1995) believe that signals reliably predicting shocks cause sensitisation of central neural control of adrenocortical activity, whereas unpredictable shocks cause habituation of the central nervous system to occur. The mechanisms behind these processes are, however, unclear.

4. Control

> Control has been defined, in relation to studies using aversive stimuli, as
>
> 'the ability to make active responses during an aversive stimulus' (Weinberg & Levine, 1980, p. 45).

Active responses may allow the animal to escape or avoid the stressor, which may have the effect of reducing stress responses. However, active responses may only allow the animal to move from one stimulus condition to another, rather than to escape from the stressor altogether. Even in this situation, an element of control appears to reduce the physiological stress response to aversive stimuli such as shock (Weinberg & Levine, 1980).

Subjects in many of the experiments conducted to investigate the effects of predictability also had a degree of control, although control was not mentioned as an experimental parameter (Weinberg & Levine, 1980). Many of the effects attributed to predictability in these studies may therefore be due to this potential confound. The 'preparatory response' hypothesis has been proposed to explain the apparent behavioural preference of animals for predictable over unpredictable shock (Perkins, 1955, 1968; Lockard, 1963). The hypothesis suggests that signals preceding events allow animals to prepare for these events, which may reduce the aversiveness of a negative stimulation, or, conversely, increase the positive nature of appetitive events (Badia *et al*, 1979). Preparation is thought to take place as a conditioned response, the biological function of which

> 'is to enable the animal to optimize interaction with the forthcoming biologically important event' (Hollis, 1982, p. 3).

For example, animals being given shocks may be able to change their posture in order to minimise these shocks.

In such experiments, what is taken to be a preference for predictable signalled shock may in fact be a preference for shock preceded by signals which enables such preparatory postural responding (Weinberg & Levine, 1980). This potential

confounding factor has however been controlled for in many studies, for example in the work of Weiss (1972), that used a tail electrode to deliver shock with varying signalled predictability to rats. This was designed specifically to prevent the problem of unequal shock, as postural movements by the animals would not displace the electrode and so reduce the intensity of shock received. Weiss (1972) found that rats exposed to unpredictable tail shocks developed more gastric ulcers than those exposed to predictable shocks, suggesting that unpredictable shocks were more stressful even when preparatory postural changes were impossible. Physiological effects of control, and the predictability-control confound, will be further discussed later in this review.

5. Feedback

An element of relevant feedback is involved in most of the experiments on predictability (Weinberg & Levine, 1980). Relevant feedback has been defined as

> 'stimuli that are not associated with the stressor and that follow a response' (Weiss, 1971a, p. 10).

Feedback provides the animal with information as to whether its response was successful in reducing or eliminating the stressor, and/or that the stressor has ceased. Animals provided with feedback, in the form of a warning tone preceding shock onset, were shown to develop fewer gastric ulcers, and therefore thought to be less stressed, than those without this information (Weiss, 1971a). Feedback is thought to be extremely important in determining animals' responses in aversive situations. Lack of feedback has been shown to increase physiological stress responses including gastric ulceration and pituitary-adrenal activity, while increased feedback may reduce these responses (Weinberg & Levine, 1980).

It has been suggested that predictability of aversive stimuli reduces stress in animals because it provides feedback about safe periods, when aversive stimuli are not likely to occur (Lockard, 1963; Seligman, 1968). This 'safety signal hypothesis' states that if an aversive stimulus, such as shock, is predicted by a signal, the absence

of that signal indicates that the situation is safe, and no shock will occur. When shock is predictable, animals will be in a state of fear only when the signal is present, and not in its absence. However, when the shock is unpredictable, there is no such safety period signalled. The animal will constantly anticipate shock, and be in a chronic state of fear. Seligman and Meyer (1970) claim that this hypothesis explains the negative physiological and behavioural effects of unpredictability.

Badia and co-workers (1976) tested whether stress was diminished only when a shock-free period was identifiable, or whether a warning signal predicting shock had the same effect. They found that animals showed no preference for dependable over undependable signals predicting shock. However, animals strongly preferred dependable over undependable 'safety' signals, which identified shock-free periods. The researchers concluded that this study strongly supported the safety-signal hypothesis, in that it showed that a dependable indicator of shock-free periods was more important to the animal than a similar indicator of shocks. Several other studies have produced results supporting the safety-signal hypothesis (*e.g.* Badia *et al*, 1971; Badia & Culbertson, 1972; Arabian & Desiderato, 1975; Hennessy *et al*, 1977). However, other studies (*e.g.* Badia *et al*, 1976) have questioned the hypothesis. It has been suggested (Badia *et al*, 1979) that preference for signalled shock may be due to a number of factors, and therefore one theory will be unable to explain all the relevant data.

As is seen with control, feedback is a factor in many experimental studies on the effects of predictability of aversive events. It has been suggested that negative aspects of unpredictability may be due to lack of information about safety, which leaves the animals in a chronic state of fear, or anticipation, as they are unable to relax preparatory responses. Positive aspects of predictability may be due to control, which has been shown to be a factor capable of reducing the physiological response of animals to stressors (Weinberg & Levine, 1980).

2.3 Experimental studies on the effects of predictability of appetitive events

It has been shown that studies investigating the effects of predictability on behavioural and physiological responses are complex, confusing and often questionable in terms of experimental validity. However, there is another important problem in the application of these findings to practical animal management and husbandry routines. Many of the experimental studies so far reported have examined the effects of predictability of aversive events. Additionally, few of the aversive stimuli used commonly occur in the life of captive animals. Common aversive stimuli might include cage cleaning or blood draws.

However, the vast majority of events in the life of captive animals are likely to be positive, such as feeding, access to conspecifics or other parts of the enclosure. It is believed (van Rooijen, 1991) that the nature (*i.e.* positive, neutral or negative) of the stimuli in question, and the context in which they are offered, will affect the consequences of predictability or unpredictability. The unquestioning generalisation of theory from studies using aversive stimuli is therefore dubious and not of direct relevance to common practice. Feeding is an event that is likely to be of great positive significance in the routine of a captive animal. Several studies have been carried out to investigate the effects of manipulating the predictability of food provisioning.

Early studies showed that pigeons (Wyckoff, 1952) and rats (Prokasky, 1956) showed a consistent preference for conditions in which they were able to use signals to predict the presence or absence of food, compared with conditions where food delivery could not be anticipated. This was despite the fact that the average amount of food delivered in each condition was the same. Prokasky (1956) suggested that the preference for predictable over unpredictable food delivery might be due to the enabling of preparatory responses, such as salivation, to occur when food delivery could be anticipated. When electric brain stimulation was used as a reinforcer, it was also found that rats preferred signalled over unsignalled reinforcement (Cantor &

LoLordo, 1970). The 'preparatory response' hypothesis, already mentioned in relation to animals' apparent preference for signalled over unsignalled shock (Perkins, 1955, 1968; Lockard, 1963) is also applicable to appetitive stimuli. It is thought that preparation for positive events may increase the reinforcing nature of such events (Badia *et al*, 1979). For example, when applied to feeding, a signal allowing the animal to predict food delivery might allow it to salivate. Food plus salivation is thought to be more reinforcing than food in the absence of salivation. Similarly, when food is not delivered, no anticipatory salivation plus no food is more reinforcing than salivation plus no food (Perkins, 1955, 1968; Badia *et al*, 1979).

Shepherdson and co-workers (1993) changed the feeding routine of three leopard cats *(Felis bengalensis)* to an unpredictable temporal schedule, and also made the food spatially unpredictable by hiding it in various places around the enclosure. Stereotypic behaviour was consequently reduced, exploratory behaviour increased and a greater range of behaviour was seen. All these changes were interpreted as being beneficial for welfare. Many similar environmental enrichment techniques use the fact that food is naturally reinforcing and interesting to animals to stimulate a range of manipulative and exploratory acts (Lindburg, 1998). Hiding food in unpredictable locations appears to improve the welfare of animals by eliciting such behaviours.

Other studies have addressed the question of whether feeding on an unpredictable time schedule may improve welfare. Studies have shown a variety of species to possess the ability to estimate time intervals (Richelle & Lejeune, 1980). The capability to detect, learn and use temporal information about events, stimuli, responses and rewards is thought to be a basic and adaptive aspect of animal behaviour (Higa & Staddon, 1997). This kind of information may play an important role in foraging strategies, allowing animals to estimate intervals between food availability and acquisition in a particular patch. This would further enable cost-benefit judgements to be made regarding moving to different areas that might yield more frequent food supplies (Taylor *et al*, 2002).

It is thought that the stereotypies shown by some captive animals, such as carnivores (Carlstead, 1998) and François langurs *(Trachypithecus francoisi)* (Krishnamurthy, 1994), may be related to an over-predictable feeding schedule. Highly predictable feeding routines have been linked to 'food-anticipatory activity' (FAA), characterised by increased arousal and activity, and documented in rodents, bees, fish, birds, rabbits, mammalian carnivores and some primate species including squirrel monkeys *(Saimiri sciureus)* (reviewed by Mistlberger, 1994) and chimpanzees *(Pan troglodytes)* (Bloomsmith & Lambert, 1995).

Frequently observed anticipatory activities include wheel running in rodents, unreinforced lever pressing, activity directed at the empty feeding trough, general cage activity and drinking (Mistlberger, 1994). These behaviours are thought to be classically conditioned through repeated pairings of the circadian phase with food presentation (Armstrong, 1980). FAA is generally seen in animals fed on a regular daily schedule, where food availability is restricted. However, the phenomenon has also been seen in rats fed standard lab chow *ad libitum*, but given a supplementary food of a chocolate mash that was presumably highly palatable (Mistleberger & Rusak, 1987). It is reasonable to expect that FAA might occur in other species in response to prized food items even when standard food is constantly available.

FAA may occur in relation to meals fed at any time on a 24-hour circadian schedule, even in environments lacking any variation in light, temperature, sound or any other external cues to feeding. However, in some animals, particularly primates, social cues such as human contact for food delivery and removal, are thought to be equally important (Mistlberger, 1994). Animals fed more than one meal per day may exhibit FAA before each one. However, when previously food-restricted animals are fed *ad libitum*, FAA dissipates and is generally absent after 3-4 days (Mistlberger, 1994).

Increased agonistic behaviour has been observed in captive primates including chimpanzees *(Pan troglodytes)* (de Waal & Hoekstra, 1980; Reynolds & Luscombe,

1969; Wilson & Wilson, 1968) and hamadryas baboons *(Papio hamadryas)* (Wasserman & Cruikshank, 1993) during pre-feeding periods when animals were fed on a predictable temporal schedule. Stump-tailed macaques showed significant increases in rates of self-directed behaviour, inactivity, vocalisation and abnormal behaviours prior to feeding (Waitt & Buchanan-Smith, 2001). Captive chimpanzees showed increased inactivity and coprophagy prior to feeding on a predictable temporal schedule (Bloomsmith & Lambert, 1995). Such behaviour, although differing from the arousal and activity definition of food-anticipatory activity, still indicates that the animals are in anticipation of feeding. The authors claim that

> 'it seemed that the subjects were "waiting" for the meal to be fed' (Bloomsmith & Lambert, 1995, p.71).

It has been suggested (Johannesson & Ladewig, 2000) that in a very predictable environment, animals may become locked into cycles of anticipating the regularly occurring events, while individuals in less predictable environments experience higher motivation for exploration and foraging. Of course, this may only be of benefit to welfare if the environment in which the animal is housed allows for such increased exploration and foraging. Bloomsmith and Lambert (1995) found that feeding chimpanzees on an unpredictable temporal schedule led to an increase in species-appropriate behaviour, which they suggested was indicative of improved welfare.

Bloomsmith and Lambert wrote that their results supported the proposal (Jordan *et al*, 1984) that, in the absence of control, predictability may be more stressful than unpredictability. In support of this suggestion, Jordan *et al* (1984) found that infant squirrel monkeys *(Saimiri sciureus)* showed greater physiological and behavioural stress responses on maternal separation of predictable duration than on that of unpredictable duration. However, according to a large body of evidence, it appears that predictable stimuli are *less* stressful than unpredictable stimuli in the absence of control (Weinberg & Levine, 1980). If predictability provides feedback

for the animal about the cessation or absence of the stimulation, it may aid coping even in the absence of control, as stated by the safety-signal hypothesis (Weinberg & Levine, 1980).

In the study by Bloomsmith and Lambert (1995), although chimpanzees received food on an unpredictable temporal schedule, they did receive a certain amount of information relating to food delivery. Food was delivered to the different groups of chimpanzees by keepers standing at the roof level of the enclosures, who were visible to some members of the colony. These animals tended to respond with food vocalisations, which acted as signals to the rest of the colony that food delivery was imminent. Therefore, although temporal predictability was low, signalled predictability was often high, although this variable was not considered by the authors. The benefits attributed to low temporal predictability may therefore have been affected by the feeding-related signals available to the animals.

Carlstead (1986) manipulated the signalled predictability of feeding by changing the reliability of signals (in the form of a bell) announcing the arrival of food to pigs. Food was delivered, from an automated hopper, on an unpredictable temporal schedule with the bell being the only information available to the pigs concerning the onset of feeding. The animals initially received food preceded by reliable signals. However, when these signals became unreliable, low predictability was found to be associated with frustration, which led to aggression and increased competition for food.

In a second experiment, pigs consistently receiving unreliable feeding signals showed a significant increase in aggressive interactions, mainly following unexpected disturbances in the environment. The author suggests that this increase was because pigs exposed to unreliable feeding signals treated these unexpected environmental noises as potential feeding signals. The failure of these 'signals' to be followed by food led to increased frustration and aggression. This did not occur in pigs which received reliable signals, however, as there was only one unmistakable signal

associated with feeding, and therefore unexpected noises were not responded to as unreliable indicators of feeding time (Carlstead, 1986). The results of this study may be viewed as in accordance with the 'safety-signal hypothesis' (Seligman, 1968; Seligman & Meyer, 1970). The unambiguous, reliable feeding signal provided information for the pigs regarding 'safe' periods when the intensely stimulating event, feeding, would not occur. Carlstead claims that feeding animals on a predictable temporal schedule will not provide enough information for them regarding 'safe' period; she states that 'the presence of an unmistakable signal is the important factor for predictability' (p. 35). It is unclear from this study whether pigs experiencing a loss of signalled predictability experienced more frustration than those that had been exposed to unreliable feeding signals from the start of the study (Carlstead, 1986).

2.4 Effects of loss of predictability on animal welfare

This review has described studies showing that deleterious behavioural and physiological consequences may occur in animals exposed to unpredictable aversive events, such as shock. However, some researchers (*e.g.* Mineka & Kihlstrom, 1978; Tsuda *et al*, 1984) have suggested that loss of predictability might produce more severe effects in animals that have had prior experience with predictable shock than in individuals that have never been exposed to predictable stimuli. In other words, loss of predictability would be more detrimental to welfare than lack of predictability (unpredictability). This hypothesis is largely due to extrapolation of findings from studies indicating that loss of control over an aversive outcome, in animals that have previously been able to control it, is more stressful than never having had control (Hanson *et al*, 1976; Selgman, 1975; Tsuda *et al*, 1983; Weiss, 1971b). However, there was no difference in the amount and severity of gastric lesions shown by rats exposed to a loss of predictability of shock, compared to those that were continuously exposed to unpredictable shock (Tsuda *et al*, 1984). These results suggest that loss of controllability of shock is more deleterious in terms of stress and gastric pathogenesis

than is loss of predictability. In contrast to this, Waitt and co-workers (2001) found that delayed cleaning routines, in which a previously predictable event became unpredictable, resulted in increases in agonistic and abnormal behaviours in stump-tailed macaques *(Macaca arctoides).* Captive primates are thought not to habituate to daily cage cleaning, which is considered to be at least mildly stressful to them (Line *et al*, 1991). Aggressive and abnormal behaviours are thought to increase in situations associated with tension and frustration (Arnone & Dantzer, 1980; Castles & Whiten, 1998; de Monte *et al*, 1992). The study by Waitt and co-workers (2001) therefore supports the idea that loss of predictability of an aversive event, caused by delays to an otherwise predictable routine, may be stressful to animals.

In an early study by Shenger-Krestovnikova (described by Pavlov, 1927) a hungry dog was given food in the presence of one conditioned visual stimulus (CS) (a circle), but not in the presence of another CS (an ellipse). The circle was therefore a reliable signal that food would be delivered. The shapes were manipulated so that the ellipse became more circular, which made discrimination between the two shapes progressively more difficult. Eventually the dog was unable to predict reliably whether the CS would be followed by food, and food delivery was therefore unpredictable. Behavioural changes such as squealing, wriggling and violent barking were seen at this point in the study. Pavlov describes it as presenting

> 'all the symptoms of a condition of acute neurosis' (p. 291).

When the discrimination between the two shapes was made easy again, the behavioural disturbances disappeared. Mineka and Kihlstrom (1978) speculate that the important variable resulting in this behavioural disturbance was loss of predictability of the food delivery in an animal that had once possessed it. The Shenger-Krestovnikova study clearly suffers from many flaws, such as a small sample size (as n=1). However, it does suggest that loss of predictability, as opposed to lack of predictability, of an appetitive stimulus may have severe consequences for animal welfare.

It has been proposed that the emotion of 'hope' is elicited by situations previously paired with pleasure (Mowrer, 1960). However, when the expected reward is not delivered, the motivation of hope is aroused but not fulfilled. This results in the aversive state of 'disappointment' (Adelman & Maatsch, 1956; Wagner, 1959). Disappointment, as a result of changes in reinforcement contingencies, results in the activation of the hypothalamic-pituitary-adrenal (HPA) system, which may be measured (Levine *et al*, 1972). Thirsty rats were trained to press a lever on a variable interval (unpredictable) or a fixed interval (predictable) schedule in order to receive a single reinforcement, consisting of water (Levine *et al*, 1972). The conditions were then switched around. Rats experiencing an unpredictable schedule that had previously been exposed to a predictable schedule (*i.e.* experiencing a loss of predictability) showed a significant elevation of plasma corticoids, thought to be indicative of frustration. In contrast, those that were changed from an unpredictable to a predictable schedule did not show increased HPA activity.

Although it is debatable whether loss of predictability of an aversive event is more stressful than lack of predictability, loss of predictability of an appetitive event may be more deleterious to welfare than constant unpredictability. This would have important implications for welfare if, for example, animals accustomed to a predictable feeding schedule experience disruptions to this routine so that feeding is delayed.

Dairy calves, fed on a temporally predictable schedule, showed various behavioural deviations when exposed to a three-hour delay to feeding (Johannesson & Ladewig, 2000). These behavioural changes were attributed by the authors to frustration when the calves' expectations were not fulfilled. Stump-tailed macaques showed increases in self-directed, agonistic and abnormal behaviours when their first meal of the day, consisting of a single piece of fruit, was delayed, representing a loss of temporal predictability (Waitt *et al*, 2001). This was despite the fact that there was still generally a considerable amount of food remaining scattered in the wood chip

floor covering from the previous day. As stated earlier, increases in these behaviours are thought to indicate tension or frustration (Arnone & Dantzer, 1980; Castles & Whiten, 1998; de Monte *et al*, 1992). The same animals showed increases in rates of self-directed behaviours, inactivity, vocalisations and abnormal behaviours prior to receiving their main feed in the afternoon (Waitt & Buchanan-Smith, 2001). However, there was no significant difference in rates of these behaviours when feeding was on time compared with when it was delayed. These results are contradictory, as a significant increase in self-directed, agonistic and abnormal behaviours was found when the first feed of the day was delayed, yet a delay in the second feed was not associated with a similar significant increase in these behaviours. However, even if delays do not cause an increase in stress-related behaviours, delayed feeding may still result in reduced welfare as these behaviours were prolonged in the second study when feeding was behind schedule. Delayed feeding therefore may or may not result in higher levels of stress for captive animals, but it is likely to cause the inevitable tension associated with feeding on a predictable schedule to be extended.

Waitt and Buchanan-Smith (2001) suggested that the negative consequences of delays may not just be due to a loss of temporal predictability, but also to the loss of reliability of external signals accompanying daily husbandry routines. When delays occur, signals such as the sounds of food preparation may still be perceived yet not followed by the expected feed. In such cases there is also a loss of signalled predictability, which may result in behavioural frustration, as was found in pigs exposed to unreliable feeding signals by Carlstead (1986). However, as yet, no studies have separated the effects of signalled and temporal predictability of appetitive events in an attempt to tease apart their relative importance to animals.

2.5 Effects of control on animal welfare

Control is thought to be psychologically and physiologically important to animals (Chamove & Anderson, 1989; Hanson *et al*, 1976; Mineka *et al*, 1986;

Overmier *et al*, 1980). An event is deemed controllable if there is a difference in the likelihood of it occurring depending on an animal's behaviour (Overmier *et al*, 1980; Sambrook & Buchanan-Smith, 1997). Experimental evidence suggests that animals have a preference for control (Overmeier *et al*, 1980). Deer mice *(Peromyscus maniculatus)*, which have an aversion to bright lighting, will use a lever to turn off a light when it comes on automatically at regular intervals (Kavanau, 1964). Their preference for control appears to override their aversion to bright light, as they will also turn it back on if it is automatically turned off. This indicates that having control over their environment is extremely rewarding to these animals. The degree of behavioural control that an animal has over a stressor is also thought to regulate the behavioural and physiological impact of that stressor (*e.g.* Anisman *et al*, 1981; Maier, 1984). For example, rats able to press a bar to avoid electric shock showed fewer physiological signs of stress, such as weight loss and gastric lesions than those that could not exercise control, even though the groups were yoked and received exactly the same amount of shock (Weiss, 1968). Positive behavioural and physiological changes have been found when captive primates have been given control over aspects of their environment such as food delivery (Line *et al*, 1991) or auditory stimuli (Hanson *et al*, 1976).

Control is thought to be so important to animals because it is the major adaptive aspect of their behaviour (Sambrook & Buchanan-Smith, 1997). In order to survive and reproduce effectively, an animal must exert control over what it eats, what eats (or does not eat) it, with whom it mates and so on. Captive animals inevitably experience reduced control over their environment, compared with their wild counterparts (Chamove & Anderson, 1989). This reduced sense of control may be the aspect of captivity that animals find most stressful and therefore is most detrimental to their welfare (Markowitz, 1982).

2.6 The relationship between predictability and control

Many experiments investigating the effects of predictability and control have confounded controllability with predictability (Overmier *et al*, 1980). In these studies, events that were uncontrollable also tended to be unpredictable, and those that were controllable were also predictable. Conversely, one of the reasons for the contrasting results in studies of the effects of predictability may be that many of these studies have included various opportunities for control and feedback (Weinberg & Levine, 1980).

The traditional view of control makes the presumption that control cannot be present without predictability – an event may be predicted without being controlled, but may not be controlled without also being predicted (Nickels *et al*, 1992).

> 'Control is confounded by predictability in that having control over a stimulus also means that it is predictable' (Schulz, 1976, p. 564).

The motivational consequences of predictability and control are thought to be closely related; some researchers suggest that this relationship is so intimate that a full understanding of these effects will only be achieved by examining them both together (Mineka & Hendersen, 1985). These researchers propose that:

> 'the effects of control and of prediction are so closely intertwined, both operationally and functionally, that it can be seriously misleading to try to examine one in isolation from the other' (Mineka & Hendersen, 1985, p. 496)

Several theories have been proposed to explain the relationships and interactions between predictability and controllability.

1. The effects of predictability and controllability are additive

Weiss (1971a) managed to separate the two variables, and found that absence of controllability and absence of predictability both increased the incidence of gastric lesions in the rat. The effects of uncontrollability and unpredictability also appeared to be additive in terms of this physiological measure. It is however, still unclear

whether these two variables result in additive behavioural effects (Overmeir *et al*, 1980).

2. Control is important to organisms because it provides predictability

Many effects that were initially attributed to control, may be due to the predictability inherent in many of the situations used to examine the effects of control. Averill (1973) claimed that the effects of control may in fact be due to the predictability associated with it, stating that:

> 'the factor of behavioral control is less important than the reduction of uncertainty which generally accompanies such control' (Averill, 1973, p. 288).

Burger and Arkin (1980) showed that predictability in the absence of control was as effective at reducing stress as predictability and control combined. However, other studies (reviewed by Gatchell, 1980 and Miller, 1979) indicate that in some situations, predictability without control over the onset of events does not appear to be as beneficial as predictability with control. Dess *et al* (1983) showed that at least in some situations, the effects of controllability and predictability are different and separate.

3. Predictability is important because it allows efficient control

This view, the converse of that suggested by Averill (1973), argues that preference for predictability is due to its enabling the individual to exert a degree of control over the stimulus (Biederman & Furedy, 1970, 1973, 1976; Furedy & Biederman, 1976; Lykken & Tellegen, 1974). For example, preference for signalled shock was found to emerge when rats in a shuttlebox developed the capacity to modify the shock they received by spending time on grids of the same polarity during the delivery of the signalled shock (Furedy & Biederman, 1976).

Although the three above models describe different relationships between predictability and controllability, they all make the same predictions for the outcome

of experiments designed to separate the two factors (Overmier *et al*, 1980). The first, additive, model would predict the greatest behavioural or physiological deficits to occur in animals exposed to a stimulus that could be neither predicted nor controlled. Intermediate levels of these deficits should be seen when the stimulus is either predictable or controllable. Animals that could predict and control the stimulus should show the least behavioural or physiological disruption.

If, as suggested by the second and third models, predictability and controllability can be reduced to a single common factor, the same predictions still hold. Animals exposed to stimuli that they can neither predict or control will experience the lowest levels of this single underlying factor, whereas those that can predict or control stimuli will experience intermediate levels. Animals that are able to both predict and control stimuli will experience the highest levels of the common factor. The fact that the three models generate identical predictions means that it is highly unlikely that researchers will be able to find experimental evidence to differentially support any of them (Overmier *et al*, 1980).

Another area of research has concentrated on the interactions between predictability and control, with the underlying assumption that the two are operationally separable (Mineka & Hendersen, 1984). The effects of predictability and control, once thought to be relatively simple, are now known to be

> 'extraordinarily complicated, requiring analysis at many different levels before they can properly be understood' (Mineka & Hendersen, 1984, p. 521).

The effects of predictability and control do not occur independently of each other, and it follows that the relationships among the various effects of these two factors will combine to create an even greater level of complexity.

Davis and Levine (1982) carried out an early study that touched upon the interactions between predictability and control. They claim that control is necessary to mediate the effects of predictability on plasma corticosterone levels of rats exposed

to signalled or unsignalled shocks. The results of their study showed that predictability of shock only affected this physiological measure of stress if rats receiving the shock were simultaneously performing the operant response of bar-pressing for food reinforcement (*i.e.* they were exercising control). Animals that were not performing this operant task showed no difference in corticosteroid response between the predictable and unpredictable shock groups. The explanation given by Davis and Levine for their findings was that the degree of control, provided by the operant task, interacted with the predictability variable to alter the pattern of the physiological stress response. However, in this experiment, the animals that could perform the operant response differed in training experience from those without access to this response. This confounding factor casts doubt on the results, and experiments that control for training experience are necessary to determine whether it is this, or the provision of control at the time of shock exposure, that accounts for observed changes in plasma corticosteroids (Mineka & Hendersen, 1984).

2.7 Implications for animal management

One major difference between the environments of captive and wild animals is the amount of environmental control available to them (Carlstead, 1996). It has been suggested by various behavioural theorists (*e.g.* Archer, 1976; Inglis, 1983; Salzen, 1962) that the degree to which an animal is stimulated by an event or situation is dependent on the discrepancy between its expectations of stimulation and the actual stimulation it receives. Additionally, these theorists propose that the immediate psychological goal of behaviour is to control the level of stimulation the animal receives from its surroundings. Animals in the natural, wild, environment are able to control the amount of stimulation they receive, by various behaviours such as approaching, attacking or hiding from the stimulus, until the stimulation they receive is at an acceptable level, or its expectations of the stimulation are met. They can control thermal stimulation by moving, for example, into the sun or away from the

wind. They can also satisfy appetitive motivation by, for example, seeking food or a mate (Carlstead, 1996).

In their review, Weinberg and Levine (1980) conclude that giving an animal an element of control over a predictable shock situation appears to make the situation less aversive. Although the majority of events in the lives of captive animals are likely to be positive, some events, such as cage cleaning or laboratory procedures including the giving of injections, may be perceived as being of an aversive nature. Providing animals with a degree of control over these events may reduce the stress associated with them. However, for practical reasons it is likely to be impossible to enable animals to have such control. In these situations, making these events as predictable as possible may provide a viable alternative, minimising the stress associated with a lack of control. Such predictability could provide animals with information regarding 'safety periods', when negatively perceived events would not occur, enabling them to relax rather than being in a constant state of anticipation of the events (Seligman, 1968; Seligman & Meyer, 1970).

Studies conducted during the last decade, however, indicate that rather than being beneficial for welfare, over-predictable environments may in themselves be stressful. This is thought to be because they may cause animals adapted to an unpredictable natural environment to become bored (van Rooijen, 1991; Wiepkema & Koolhaas, 1993). It has been suggested that, for optimal welfare, predictability of environmental events should be of an intermediate value, although this suggestion has not been substantiated (Novak & Drewson, 1989; Wiepkema & Koolhaas, 1993). Considerable research is necessary, however, to investigate the effects of predictability of positive, negative and neutral events in the lives of captive animals. It is also necessary to identify which events are most important to animals in terms of variations in predictability. A major challenge for research in this area is to identify optimal levels of predictability in order to enhance the welfare of captive animals.

In the captive situation, there will always be certain signals associated with feeding, such as the sound of food preparation, doors being unlocked or other animals being fed. These signals may not always be reliable, especially in situations where there are many animals and therefore many feeding-related signals. Unreliable signals may lead to frustration, but it is unrealistic to expect these signals to be eliminated. In these situations, it may be useful for animals to learn to associate a unique noise, such as a buzzer or bell, with feeding. This sound would only be heard prior to feeding, and may help to extinguish previously learned signal associations as described above, which may not be reliable. It may be possible, using such a method, to feed on an unpredictable temporal schedule and derive benefits such as those seen by Bloomsmith and Lambert (1995) (*i.e.* increased species-appropriate behaviour), but without the negative consequences observed by Carlstead (1986) (*i.e.* aggression). This hypothesis, however, remains to be tested.

2.8 Aims of the thesis

The effects, on behaviour and welfare, of predictability of appetitive events such as feeding have rarely been studied in animals in general or primates in particular. Despite this relative lack of empirical testing, recommendations have been made as to the most beneficial timing of feeding and other husbandry routines. These suggestions, for example that predictability of environmental events should be of an intermediate value to optimise well-being (Noval & Drewson, 1989; Wiepkema & Koolhaas, 1993), are largely based on anecdotal evidence and presuppositions. The broad aim of this thesis is to evaluate the effects on behaviour and welfare of captive primates, of differences in the predictability of food delivery.

In order to use behaviour to assess the effects on welfare of experimental manipulations of any kind, it is necessary to have an idea of behaviours normally associated with reduced welfare of the species in question. Despite the fact that common marmosets *(Callithrix jacchus)* (the study animal used in three of the four

experiments described here) have been used as laboratory animals for many years, there is still no clear protocol for interpretation of behavioural data in the light of welfare changes. The study presented in Chapter 4 attempts to address these issues by correlating behavioural and physiological changes in response to a mild stressor. This was intended to add validity to the use of behavioural measures as a non-invasive and relatively simple technique to assess welfare in this species.

The relationship between predictability and control is thought to be profound and complicated. However, although it may be impossible to achieve control without predictability, it is possible to have predictability without control (Nickels *et al*, 1992). Throughout the thesis, it was intended to examine the effects of predictability. In order to avoid potential confounds between predictability and control, control was not manipulated, and at no point did the animals have control over food delivery.

The predictability of a stimulus or event may be broken down into two separate components, which I attempted to tease apart in the experiments described in Chapters 5 and 6. These experiments were carried out in a very controlled environment, using titbits rather than attempting to manipulate the timing of the actual feed. The two proposed components of predictability are as follows:

(a) Signalled predictability

Preceding an event by a signal will increase its predictability if that signal is reliable (*i.e.* only occurs before the event, occurs before every event and there is the same time interval between the signal and the event). Manipulating the reliability of the signal will affect the predictability of the event.

(b) Temporal predictability

A temporally predictable event occurs either at the same time each day, or at the same time interval after another event. Temporal predictability may be manipulated by changing the timing of the relevant event in terms of the time of day at which it

occurs, or its timing after another event (such as the arrival of the experimenter, or the presentation of a signal). The event may therefore occur on either a fixed-interval or variable-interval schedule.

In addition to these two different types of predictability, a further variable became apparent, which was manipulated in the experiments in both Chapters 5 and 6, but only specifically addressed in Chapter 6. This related to the percentage of experimental trials in which a piece of food was delivered, and may be considered as a third type of predictability, albeit one pertinent to the experimental situation described in the thesis rather than the real world. This third type of predictability is described below.

(c) Predictability related to reinforcement schedule

The non-contingent reinforcement schedule of an event may be manipulated, effectively changing the event's predictability. The reinforcement schedule was non-contingent in the experiments described in this thesis as the delivery of the food was not dependent on the behaviour of the animals. With a reinforcement schedule of 100%, the food is delivered on all relevant occasions, or 100% of trials. However, when the schedule is changed to, say, 50%, food is given only 50% of occasions. The lower the reinforcement schedule, the lower the predictability of food delivery.

The experiment described in Chapter 7 attempts to relate the findings of those in Chapters 5 and 6 to a different species, the stump-tailed macaque *(Macaca arctoides)*, in a real life situation. For this study I observed behavioural changes in the animals seen around feeding time, and then introduced and manipulated the reliability of signals to change the predictability of this feed. Although feeding is clearly essential to the physical well-being of animals, its effect on psychological well-being has rarely been directly addressed (but see, *e.g.* Bloomsmith & Lambert,

1995; Carlstead, 1986; Waitt & Buchanan-Smith, 2001; Waitt *et al*, 2001 for exceptions). It is hoped that the findings of the experiments presented in this thesis will highlight the importance of the little considered variable of predictability of feeding on the welfare of captive animals. Recommendations relating to the optimal timing and predictability of husbandry routines such as feeding, which are intended to improve the welfare of captive primates and, by extension, other animals, are presented in the final discussion (Chapter 8).

Chapter 3

Methods

3.1 INTRODUCTION

Applied behavioural research in the laboratory setting

This thesis examines the effects, on the behaviour and welfare of captive primates, of variations in the predictability of feeding. Feeding is an event imposed by human caretakers on animals, and all experiments described here were conducted on captive populations. Therefore many of the problems associated with observing animals in the wild, and controlling or manipulating variables in field experiments are not relevant to these studies. Additionally, validity in relation to the natural lives of animals, often a problem with more traditional studies in animal behaviour (Dewsbury, 1994) is not so significant in this, more applied, type of research.

It might be expected that the study animals used in the experiments described here would not exhibit the full range of their natural behaviour patterns, as they were housed in unnatural social groupings in cages that were small and barren, relative to their natural environments. This would be a problem if the phenomena under consideration occurred under natural conditions and it was intended to extend conclusions between captive studies and the wild. However, the nature of the research is such that it is not intended to be generalizable to a more ecologically relevant situation. Rather, it was anticipated that the results might show generality across animals in captive conditions, an hypothesis that was tested by using two species, housed in different enclosures and social groupings.

Types of experimental animal behaviour research may be viewed as lying along a continuum. At one end is the ecologically valid field experiment, which is conducted under natural conditions but which is often difficult to carry out, control and interpret. At the other extreme is the artificial and strictly controlled laboratory study, which may be easier to control, but may also fail to stimulate the full range of

behavioural responses that may be seen in the wild. The experiments described in Chapters 4-6 lie towards this end of the continuum. Somewhere between these two extremes is the study carried out in captivity but based on manipulation of factors that occur in the life of the captive animal, such as that described in Chapter 7.

The experiments carried out to examine the effects of feeding predictability in marmosets (Chapters 5 and 6) were highly controlled so that any effects might be easily detected, with extraneous variables removed as far as was possible. These studies were, however, extremely artificial. For example, predictability of the real feed was not manipulated, a desirable titbit being substituted instead. Additionally, the time scales involved were very short (data collection only lasting for eight minutes per session) and this may have resulted in misleading conclusions, if attempts were made to extrapolate the results to real husbandry routines. The macaque study (Chapter 7) was therefore intended to see whether the conclusions drawn from the marmoset experiments were valid in a real-life situation.

Behavioural testing in the captive environment enables manipulation of experimental variables under controlled conditions. This helps to reduce the number of plausible alternatives that could explain the outcome (Martin & Bateson, 1986). The studies with the marmosets in this thesis were designed so that different study animals were used in each condition. This is known as a between-subjects design. The age, sex and history of individuals were known and were matched across conditions to reduce potentially confounding influences on the results. The large numbers of animals available resulted in reasonable sample sizes in each experiment. This was advantageous as increasing sample size increases statistical power, which in turn increases the probability of detection of a real effect.

Despite the fact that captive studies enable matching of study animals between conditions, individual differences may be considerable. These may result in differences in the responses of one animal compared to another in an identical condition, and lead to large variability in the data (Howell, 1989). The easiest way

to reduce this potentially confounding factor is to use a repeated-measures design, when each individual effectively acts as its own control. This is also the only method to use when the number of available study animals is not large enough to enable different animals to take part in each condition. This was the case with the macaque study described in Chapter 7. The repeated-measures design is more powerful than the between-subjects design, thus increasing the probability of rejecting a false null hypothesis (H_0) (Howell, 1989).

A major problem with this approach is that each treatment may have a lasting effect, which may affect results of subsequent treatments. This is generally countered by using a random order of treatments for each study animal. However, this was not possible in the experiment described in this thesis. The design of the building was such that all groups could hear the feeding-related sounds that were manipulated in each experimental treatment. The inability to control all factors, such as order effects, is one of the problems encountered when moving along the continuum from an entirely artificial experiment to one in a more real-life setting. This factor should be borne in mind when interpreting the results.

3.2 STUDY ANIMALS

Study animals were common marmosets *(Callithrix jacchus)* (Plate 1) and stump-tailed macaques *(Macaca arctoides)* (Plate 2). Animals were housed at the Medical Research Council (MRC) Human Reproductive Sciences Unit, Bush Estate, Edinburgh, Scotland. All animals were captive bred. These species were selected as they represent both New World (Platyrrhine) and Old World (Catarrhine) infraorders (Groves, 2001). Any consistent effects shown by such taxonomically different species would increase confidence in the generalizability of conclusions drawn. On a practical level, the availability of large numbers of these species resulted in good sample sizes for the experiments.

Plate 1 Common marmoset *(Callithrix jacchus)* carrying youngster

Plate 2 Stump-tailed macaque *(Macaca arctoides)*

3.2.1 Common marmosets

The MRC Unit maintains a large colony of common marmosets, usually numbering around 300 individuals. All the marmosets used in the study were pair-housed, with a total of 122 individuals used in three separate experiments. Ten animals took part in both of the final two studies (Chapters 5 and 6). Studies on the marmosets took place between September 2000 and August 2001. Details of the pairs used are given in Tables 3.1 – 3.3. It is standard MRC procedure for all marmosets to wear a tag worn on a chain around the neck to aid identification.

Table 3.1 Details of age and sex of common marmosets used as study animals in the study described in Chapter 4

Condition	Pair	Individual I.D.	Sex	Date of Birth	Age (days)[1]
Trained	1	843BK	M	10/09/98	861
		952R	F	15/02/99	706
	2	832BK	M	18/07/98	913
		895R	F	18/07/98	913
	3	878BK	M	09/07/99	562
		946R	F	01/02/99	720
	4	683BK	M	10/05/94	2421
		975R	F	29/07/99	542
	5	833BK	M	19/07/98	912
		829R	F	22/06/97	1299
	6	813BK	M	11/05/98	980
		902R	F	22/07/98	909
Untrained	7	864BK	M	22/04/99	639
		678R	F	29/03/93	2822
	8	878BK	M	24/06/99	577
		971R	F	24/06/99	577
	9	788BK	M	24/11/97	1147
		902R	F	22/07/98	909
	10	870BK	M	04/05/99	627
		685R	F	15/06/93	2746
	11	804BK	M	15/03/98	1036
		909R	F	11/08/98	890
	12	802BK	M	04/03/98	1047
		940R	F	23/12/98	758

[1] As of 01/02/01, when data collection began

Table 3.2 Details of age and sex of common marmosets used as study animals in the study described in Chapter 5

Condition	Pair	Individual I.D.	Sex	Date of Birth	Age (days)[2]
A	1	911R	F	24/08/98	727
		741BK	M	03/09/98	718
	2	733R	F	13/02/95	1998
		746BK	M	01/07/96	1500
	3	926R	F	09/10/98	682
		959R	F	12/04/99	499
	4	990R	F	30/11/99	271
		897R	F	20/07/98	761
	5	781BK	M	21/08/97	1090
		830BK	M	07/07/98	774
	6	868BK	M	29/04/99	482
		889BK	M	31/10/99	301
B	7	729R	F	25/07/94	2196
		812BK	M	11/05/98	830
	8	967R	F	26/05/99	455
		692BK	M	14/06/94	2237
	9	927R	F	12/10/98	679
		938R	F	10/12/98	621
	10	944R	F	26/01/99	575
		954R	F	02/03/99	539
	11	803BK	M	15/03/98	886
		840BK	M	24/08/98	727
	12	737BK	M	29/03/96	1592
		867BK	M	09/04/99	502
C	13	823R	F	16/08/98	735
		729BK	M	25/02/96	1626
	14	861R	F	10/12/97	981
		649BK	M	15/02/93	2716
	15	875R	F	29/03/98	872
		948R	F	09/02/99	562
	16	925R	F	09/10/98	682
		951R	F	15/02/99	556
	17	697BK	M	20/06/94	2231
		805BK	M	05/04/98	866
	18	862BK	M	09/04/99	502
		844BK	M	30/09/98	691
D	19	855R	F	07/11/97	1014
		820BK	M	16/06/98	795
	20	903R	F	22/07/98	759
		669BK	M	01/11/93	2460
	21	945R	F	26/01/99	575
		992R	F	15/07/99	406
	22	955R	F	02/03/99	539
		983R	F	27/09/99	334
	23	809BK	M	19/04/98	852
		825BK	M	26/06/98	785
	24	785BK	M	07/11/97	1014
		842BK	M	10/09/98	711

[2]As of 01/09/00, when data collection began.
Animals shown in red were also used in the study described in Chapter 6.

Table 3.3 Details of age and sex of common marmosets used as study animals in the study described in Chapter 6

Condition	Pair	Individual I.D.	Sex	Date of Birth	Age (days)[3]
A	1	970R	F	24/06/99	697
		891BK	M	13/02/00	468
	2	960R	F	19/04/99	762
		806BK	M	07/04/98	1134
	3	985R	F	06/10/99	595
		998R	F	25/02/00	456
	4	897R	F	20/07/98	1031
		990R	F	30/11/99	541
	5	658BK	M	03/07/93	2848
		814BK	M	20/05/98	1091
	6	765BK	M	13/02/97	1548
		901BK	M	21/03/00	430
B	7	830R	F	22/06/97	1419
		836BK	M	21/07/98	1030
	8	855R	F	07/11/97	1284
		820BK	M	16/06/98	1065
	9	978R	F	19/08/99	642
		997R	F	25/02/00	456
	10	29Y	F	01/08/00	300
		982R	F	26/09/99	605
	11	846BK	M	09/11/98	922
		919BK	M	01/08/00	300
	12	783BK	M	30/09/97	1321
		902BK	M	22/03/00	429
C	13	20Y	F	02/06/00	359
		879BK	M	20/07/99	671
	14	992R	F	27/12/99	514
		897BK	M	28/02/00	453
	15	984R	F	04/10/99	597
		4Y	F	06/03/00	445
	16	905R	F	31/07/98	1021
		996R	F	25/02/00	456
	17	889BK	M	31/10/99	571
		931BK	M	21/09/00	250
	18	784BK	M	10/10/97	1311
		921BK	M	01/08/00	300
D	19	850R	F	10/10/97	1311
		877BK	M	24/06/99	697
	20	999R	F	27/02/00	454
		840BK	M	24/08/98	997
	21	921R	F	10/09/98	981
		993R	F	27/12/99	514
	22	901R	F	21/07/98	1030
		17Y	F	05/05/00	386
	23	824BK	M	18/06/98	1063
		920BK	M	01/08/00	300
	24	649BK	M	15/02/93	2986
		915BK	M	28/06/00	333

Table 3.3 (cont.)

Condition	Pair	Individual I.D.	Sex	Date of Birth	Age (days)[3]
E	25	911R	F	24/08/98	997
		809BK	M	19/04/98	1122
	26	676R	F	25/03/93	2946
		657BK	M	03/07/93	2848
	27	922R	F	24/09/98	967
		30Y	F	01/08/00	300
	28	950R	F	12/02/99	829
		2Y	F	02/03/00	449
	29	686BK	M	11/05/94	2540
		911BK	M	05/05/00	386
	30	797BK	M	18/02/98	1183
		837BK	M	22/07/98	1029

[3] As of 01/06/01, when data collection began
Animals shown in red were also used in the study described in Chapter 5

3.2.2 Stump-tailed macaques

A total of 31 stump-tail macaques were held by the MRC at the time of the experiment. A large breeding group, consisting of a male, several breeding females and their offspring (thirteen individuals in total) was not included in the study. This was because it was felt that differences in group size and composition might affect the results. Study animals were eighteen adults, housed in five separate groups in structurally identical enclosures. Groups ranged in size from three to five individuals. Two groups were all-female, while the other three each contained one male and between two and four females. Groups contained adult animals only. See Table 3.4 for details of group compositions. Data collection for the macaque study took place between April and October 2001. The macaques were not marked or tagged to aid identification. Individuals were easily recognisable by body size and shape, as well as facial and body markings.

Table 3.4 Details of age and sex of stump-tailed macaques used as study animals in the study described in Chapter 7

Group	Individual	Sex	Date of birth	Age (years)[4]
1	Gail	F	04/07/79	21
	Penny	F	15/05/83	17
	Maureen	F	04/01/85	16
2	Cedric	M	17/05/92	8
	Carol	F	24/11/82	18
	Ruth	F	13/10/85	15
3	Jill	F	04/10/76	24
	Fingers	F	07/12/87	13
	Face	F	24/05/89	11
4	Wilmot	M	29/01/94	7
	Cola	F	01/05/82	18
	Bep	F	31/08/80	20
	Wendy	F	03/10/79	21
5	Blacky	M	unknown	≈20
	Jane	F	02/01/83	18
	Miriam	F	13/08/86	14
	Noreen	F	25/01/92	9
	Kelly	F	07/12/94	6

[4] As of 01/04/01, when data collection began

3.3 HOUSING AND HUSBANDRY

All observations of the marmosets took place in their home cages, and those of the macaques in their gang rooms. This ensured that moving the animals to a strange environment did not disrupt their behaviour. Housing and husbandry routines of the two species were very different, and are therefore described separately.

3.3.1 Housing

(a) Common marmosets

Marmosets were housed in cages measuring approximately 0.65 m wide by 1.15 m high by 1.1m deep. Cages were furnished with branches to facilitate climbing, a metal nest-box and a rubber matting 'shelf' attached to the mesh of the cage front about two-thirds of the way up. The cage floors were covered with a thick layer of wood shavings to enable foraging (see Plates 3 and 4), and water was

available *ad libitum* from a bottle mounted on the front mesh of the cage. Each cage was made up of one quarter of a larger cage (these were used, without being divided, to house family groups) (see Plate 5), and could be either 'upper-tier' or 'lower-tier'. Animals in upper- and lower-tier cages were balanced between experimental conditions as it has been suggested that the position of the cage may affect behaviour and welfare. It is thought that animals in lower-tier cages may experience reduced welfare, because the lower cage is darker (Scott, 1991) and animals housed therein may receive less attention from laboratory staff as it is less convenient for staff to bend down to carry out husbandry procedures (Buchanan-Smith *et al*, 2002). Animals are also unable to reach heights above those of humans when performing the vertical flee response, which may be especially detrimental for arboreal species such as the common marmoset that may experience stress associated with an enforced terrestrial lifestyle (Reinhardt & Reinhardt, 1999c).

Many of the lower tier cages were fitted with mesh 'verandahs', which enabled animals to come out and be in visual contact with their neighbours in adjacent cages (see Plate 5). Large cages were positioned side by side along opposite walls of the 'colony rooms' (see Plate 6), which gave individuals visual contact with animals on the opposite side of the room. There were eight large cages, four along each of two opposite walls, and hence up to 32 pairs of marmosets in each colony room (see Figure 3.1). Marmosets were able to hear, and also occasionally to view, laboratory staff going about their routines as windows were present in the top half of the door to the colony rooms. The temperature in the marmoset rooms was maintained at 22-23°C and humidity at around 55%.

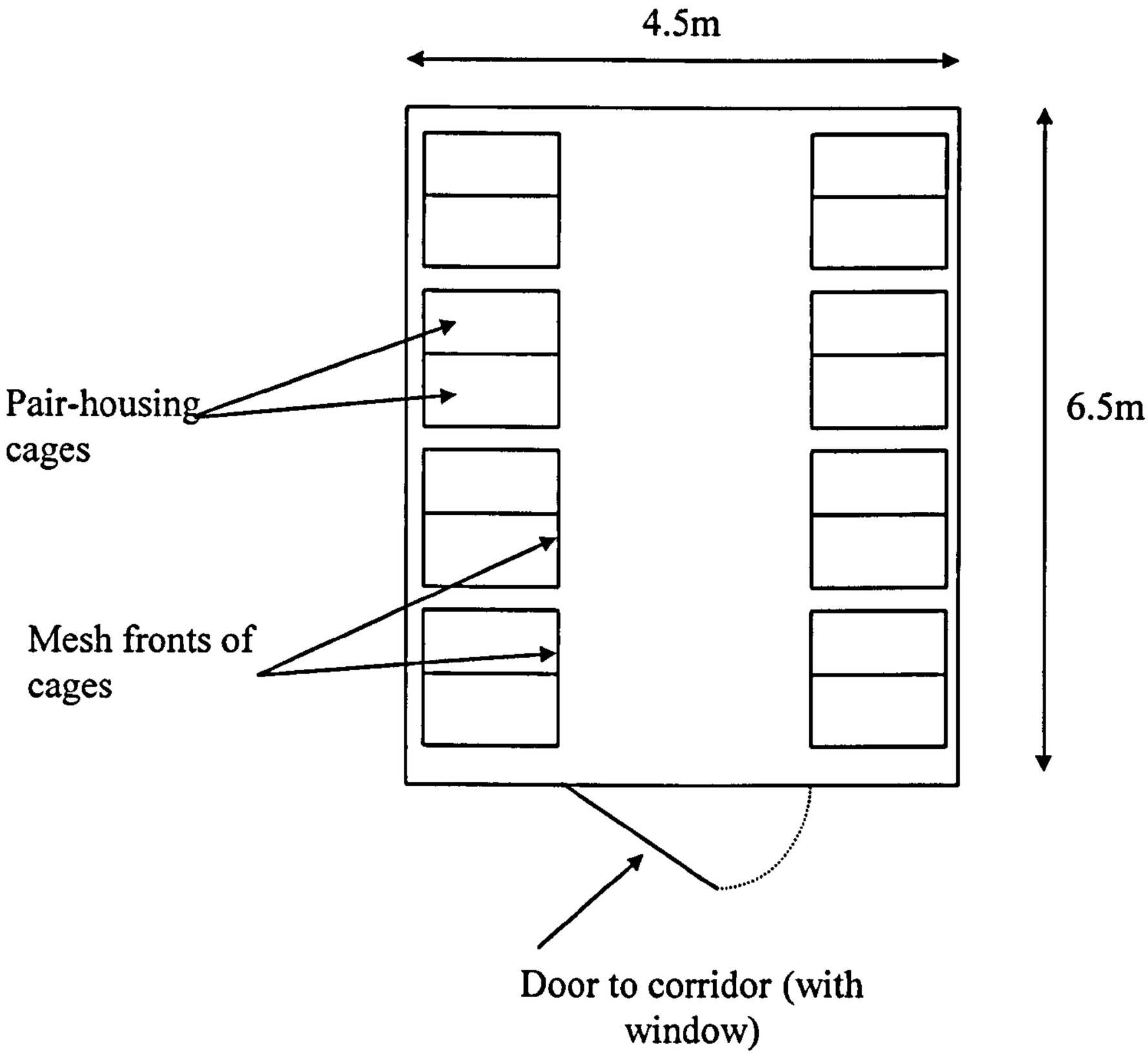
Plate 3 Marmosets in pair-housing cage with door closed

Plate 4 Marmosets in pair-housing cage with door open

Plate 5 Large cage split into four pair-housing cages ('Verandah' visible on bottom right-hand portion)

Plate 6 Bank of cages along one wall of marmoset 'colony room'

Figure 3.1 Schematic diagram of marmoset colony room (not to scale)



(b) Stump-tailed macaques

Housing for the macaques consisted of indoor enclosures, known as 'gang rooms', measuring 2.7 x 2.8 x 4.8m. These were furnished with wooden and metal structures to facilitate climbing and provide perches (see Plate 7). The floors of the enclosures were covered in a deep layer of wood shavings, amongst which meals were scattered, to provide opportunities for foraging (Plate 8). Large windows (1 x 0.88m) were present on the front wall of the enclosures. These gave the observer unobstructed visual access to the whole room, as well as enabling the monkeys to view the technicians' access corridor adjacent to their enclosures (Plate 9). Each group also had access to two tiers of cages in an adjacent room, situated along opposite walls (Plate 10). Cage rooms each contained banks of cages for two separate groups; in these rooms animals might therefore have visual and auditory, but not tactile, access to members of other groups. The observer did not have visual access to the animals when they were in the cage room. See Chapter 7 (page 255) for schematic diagram of macaque housing.

3.3.2 Husbandry routines

(a) Common marmosets

The marmoset rooms were maintained on a twelve-hour light / dark cycle. Food dishes and uneaten food were removed daily from the cages. Colony room floors were also cleaned on a daily basis, with cleaning taking place between 0800 and 1030h. Cleaning was not carried out at weekends. The wood shavings in the cages were changed weekly, and the whole cages washed in a cage washing machine once a month.

Water was available *ad libitum*, and marmosets were fed once daily, on a temporally consistent schedule, at around 1300h. Food consisted of a mixture of New World primate pellets, fresh and dried fruit (e.g. apples, pears, oranges, tomatoes, grapes, dates and raisins) and peanuts. Three times a week, they were

Plate 7 Macaque in 'gang room'

Plate 8 Macaques foraging amongst wood shavings

Plate 9 Macaques watching human activity through viewing window, from where they were observed

Plate 10 Bank of cages along one side of macaque 'cage room'

also given marmoset 'porridge', consisting of yoghurt, baby rice and protein mixture with added vitamins and minerals. Food was prepared into paper 'dishes', which were placed on the cage floor. Any routine experimental or husbandry procedures, such as blood sampling, weighing or manual palpation to detect pregnancy, were generally carried out in the morning.

(b) Stump-tailed macaques

Cleaning took place in the morning, generally between 0830h and 1030h. Gang rooms were cleaned out twice a week, when new wood shavings were put down. Cage rooms were dry cleaned daily, and power hosed twice a week. No cleaning was carried out at weekends. Any routine experimental procedures (blood sampling or vaginal swabbing, which were only performed on adult females) were carried out in the morning. Females were trained to present for swabbing, and to offer a limb for blood collection. These procedures were carried out in order to monitor reproductive cycling. Afternoons were maintained as 'quiet time' for the monkeys. Water was available *ad libitum* and animals were fed once a day on a fairly consistent temporal schedule, generally between 1230h and 1330h.

Food consisted of a mixture of Old World primate pellets, fresh fruit and vegetables (e.g. apples, oranges, bananas, cabbage, cucumber and lettuce). About twice a week, 'extras', such as yoghurts, were also given at random times. Food was scattered on the floor of the gang rooms, amongst the wood shavings and on the wooden perches, to maximise foraging opportunities.

3.4 OBSERVATION PROTOCOL, DATA COLLECTION AND SAMPLING METHODS

3.4.1 Observation protocol

(a) Common marmosets

All data collection took place when the marmosets were in their home cages. During data collection, I stood (in the case of animals in upper-tier cages) or sat (lower-tier cages) around 1.5 metres from the front of the cage. My head was therefore approximately level with the centre of the cage, regardless of whether it was upper- or lower-tier. This was thought to be important as changes in behaviour have been found in a variety of zoo-housed primate species when an audience was asked to crouch, as opposed to standing, in front of their cage (Chamove *et al*, 1988). It also gave me a good view of the marmosets, which were only out of sight when in the nest-box. I remained as still as possible, only moving towards the cages to deliver pieces of food. However, habituation to my presence never completely occurred. All animals tended to spend considerable amounts of the data collection periods inactive at the front of the cage, watching me. It was for this reason that two sub-categories of 'inactivity' were initially used, 'inactive (watching observer)' and 'inactive (not watching observer)'. It was not considered practicable to use a hide as the animals tended to retreat into their nest boxes when one was introduced. The monkeys did not appear to habituate to the presence of the hide even when it was left in place for relatively long periods.

(b) Stump-tailed macaques

All data collection took place when animals had free access to both their gang rooms and cages. I stood directly in front of the viewing window (see Plate 9), which enabled me to see the whole of the gang room. However, I could not see the animals when they were in the cage room. At these times they were recorded as

'out of view'. All animals seemed to be well habituated to my presence, and appeared to pay me no attention.

All data relevant to the study were collected just before and after the daily feed was given. I was interested in the effects of manipulating predictability of feeding, and my presence might have become a cue that feeding was about to occur. I therefore also watched the animals at various other times of the day. It is still possible, however, that I was perceived as a feeding cue, albeit an unreliable one. I will return to this point in the relevant chapter.

3.4.2 Data collection

(a) Hand-held computer

A hand-held computer running THE OBSERVER 3.0 event recording computer programme (Noldus, 1993) was used for data collection in all studies. This enabled behavioural observations to be directly entered into the computer, and recorded, via the keyboard. The use of this medium was advantageous in that it enabled data to be more quickly entered and transcribed into a usable form for analysis than would have been possible with traditional check sheets. The computer was also useful in that it acted as a stopwatch and metronome for time sampling. After a set time interval, it emitted a tone, the volume of which could be controlled so as to be audible yet to disturb the animals as little as possible.

However, the computer programme used was not as adaptable as check sheets, in that it was not possible to record instantaneous data at the same time as data collected on an all-occurrences basis. The computer and check sheets were used simultaneously in the instances when I thought that both types of data might be useful. Pilot trials were run until I felt that I was competent in keying in data into the computer.

(b) Checksheets

Check sheets were used in one study (described in Chapter 4) to record all occurrences of behaviours with a short duration, such as scratching and scent marking. Scan sampling, with a time interval identical to the one used for data collected on the hand-held computer, was used. Check sheets were designed according to recommendations made by Martin and Bateson (1986).

3.4.3 Sampling methods

Scan sampling was used in all the experiments described in this thesis. This was in order that all individuals might be observed in each session, yet bias associated with *ad libitum* sampling might be minimised. When the *ad libitum* sampling method is used, behaviours performed by individuals that are less conspicuous may be under-recorded (Martin & Bateson, 1986). Additionally, especially with the larger macaque groups, it was considered to be inappropriate to use *ad libitum* sampling, as many important behaviours might have been missed, it being impossible to keep an eye on four or five individuals and enter data simultaneously.

Scan sampling resulted in data in which all individuals were evenly represented. The mutually exclusive behavioural categories that were used for each species are presented in Tables 3.5 and 3.6. The behavioural categories were decided following a short pilot study. Behaviours that were seen at very low frequencies (*e.g.* 'inactive, inalert' and 'allogrooming' in the marmoset and 'copulation' in the macaque) were included in an 'other' category.

The recording rule used was instantaneous time sampling (Martin & Bateson, 1986). The sampling interval was kept fairly short (15 seconds for the marmoset studies and 30 seconds for the macaques) to reduce the probability that behaviours of a short duration would be missed. Data points collected with such a short interval between scans may not be statistically independent of each other

(Martin & Bateson, 1986). However, as single scores for the analyses were derived from means of the individual data, independence of data points was not a problem in the experiments described in this thesis.

Table 3.5 - Mutually exclusive behavioural categories and definitions used for common marmosets (Descriptions of scent marking based on Stevenson & Poole, 1976)

Behavioural category	Definition
Inactive (watching observer)	Animal remains in one location, without engaging in any other activity, whilst watching observer
Inactive (not watching observer)	Animal remains in one location, without engaging in any other activity. Does not watch observer, but looks at the surroundings or other individual. Inalert behaviour was included in this category
Locomote	Animal moves between locations by walking, climbing, running or jumping
Self-scratch	Animal scratches itself with a hand or foot
Scent mark	Animal sits and rubs anogenital area on branch or other area of enclosure (anal scent mark), or rubs sternal area along substrate (sternal scent mark)
Vocalise	Animal emits any kind of vocalisation audible to observer. Animal must also be seen to vocalise for this behaviour to be scored. In cases where the animal performed this and another behaviour, *e.g.* locomotion, simultaneously, 'vocalise' took priority.
Forage	Animal is engaged in any activity directly related to acquiring or ingesting food
Other	Any behaviour not otherwise listed (*e.g.* allogrooming)

Table 3.6 Mutually exclusive behavioural categories and definitions used for stump-tailed macaques

Behavioural Category	Definition
Abnormal	Animal engages in apparently functionless behaviour such as eye poking, hair plucking, self aggression, self clasping, rocking, coprophagia or wall licking
Affiliative	Animal is grooming, being groomed by, huddling, embracing, presenting to or playing with another
Agonistic	Animal is behaving aggressively or submissively; *e.g.* chasing, threatening (physically or vocally), biting, slapping, looking away or being displaced
Autogroom	Animal grooms itself with hands, feet or mouth
Forage	Animal is engaged in any activity directly related to acquiring or ingesting food or water
Inactive alert	Animal remains in one location without engaging in any other activity. Looks at surroundings, observer or other individuals
Locomote	Animal moves between locations by walking, climbing, running or jumping
Self-scratch	Animal scratches itself with a hand or foot
Vocalise	Animal emits any kind of vocalisation audible to the observer. In cases where the animal performed this and another behaviour, *e.g.* locomotion, simultaneously, 'vocalise' took priority.
Other	Any behaviour not otherwise listed (*e.g.* copulation)

In order to establish whether it was valid to use instantaneous sampling, albeit with a short scan interval of 15 seconds, to record frequencies of behaviours with a short duration (*i.e.* self-scratching, scent marking and vocalising), a correlation was carried out. This examined the relationship between frequencies of behaviours recorded by instantaneous sampling with those recorded using the more

exact all-occurrences (continuous) recording (Martin & Bateson, 1986), and was conducted on data collected for the study described in Chapter 4 of the thesis. All data relating to these three brief behaviours for the trained animals were collected using both methods. This generated 168 data sets (14 for each of the 12 animals). In order that data points should remain statistically independent of each other, a mean for each animal was calculated from all of these data sets. Furthermore, as the behaviour of each individual may have been influenced by its cage mate and therefore could not be considered truly independent, a mean for each pair was calculated and used in a Pearson correlation. One-tailed tests were used as I had predicted that the data collected by the two methods would be positively correlated. There were significant correlations between the scores obtained by the two sampling methods for all three behavioural categories (self-scratch: Pearson $r=0.77$; $n=6$; $p<0.05$; scent mark $r=0.99$; $n=6$; $p<0.001$; vocalise $r=0.99$; $n=6$; $p<0.001$, all tests one-tailed). It was therefore considered valid to use frequencies generated from instantaneous sampling for these behaviours, and this method was used throughout the thesis. See Figures 3.2 – 3.4 for scatterplots illustrating the relationship between the two sampling methods.

It was necessary to control for potential effects of circadian rhythms on the behaviour of the marmosets, an effect that has been reported for this species by various authors (*e.g.* Erkert, 1989; Hubrecht, 1985, Menezes *et al*, 1993). Also, the marmosets were fed during the middle of the day. As they were only fed once a day, they were likely to be hungry in the morning before food was delivered. Food restriction is common in research, and is known to cause increased arousal, which may affect behaviour (review by Toth & Gardiner, 2000). Observations were therefore evenly balanced between morning (1100-1230h) and afternoon (1400-1530h) sessions.

Figure 3.2 Scatterplot of frequency of self-scratching obtained by all-occurrences recording against instantaneous sampling

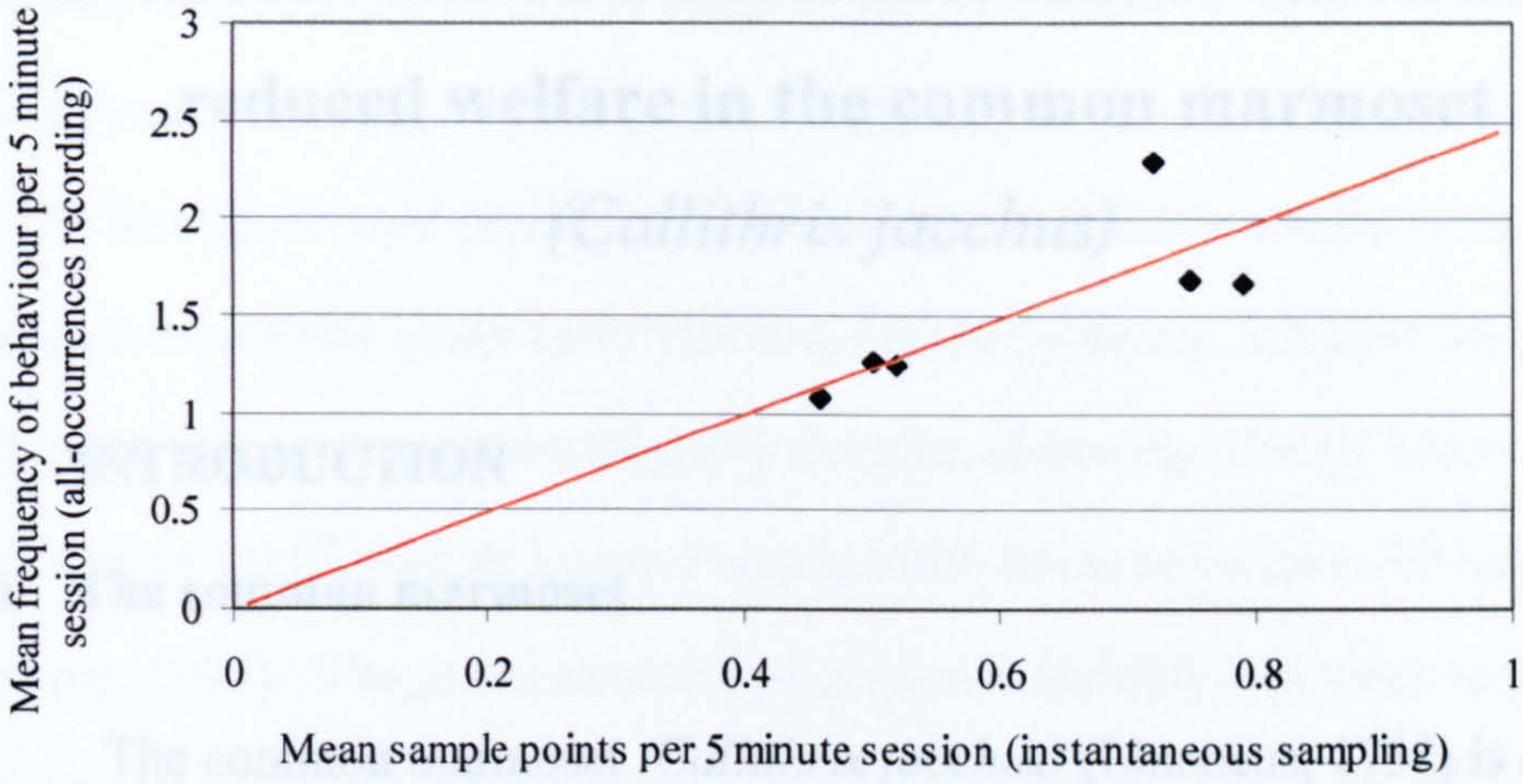


Figure 3.3 Scatterplot of frequency of scent marking obtained by all-occurrences recording against instantaneous sampling

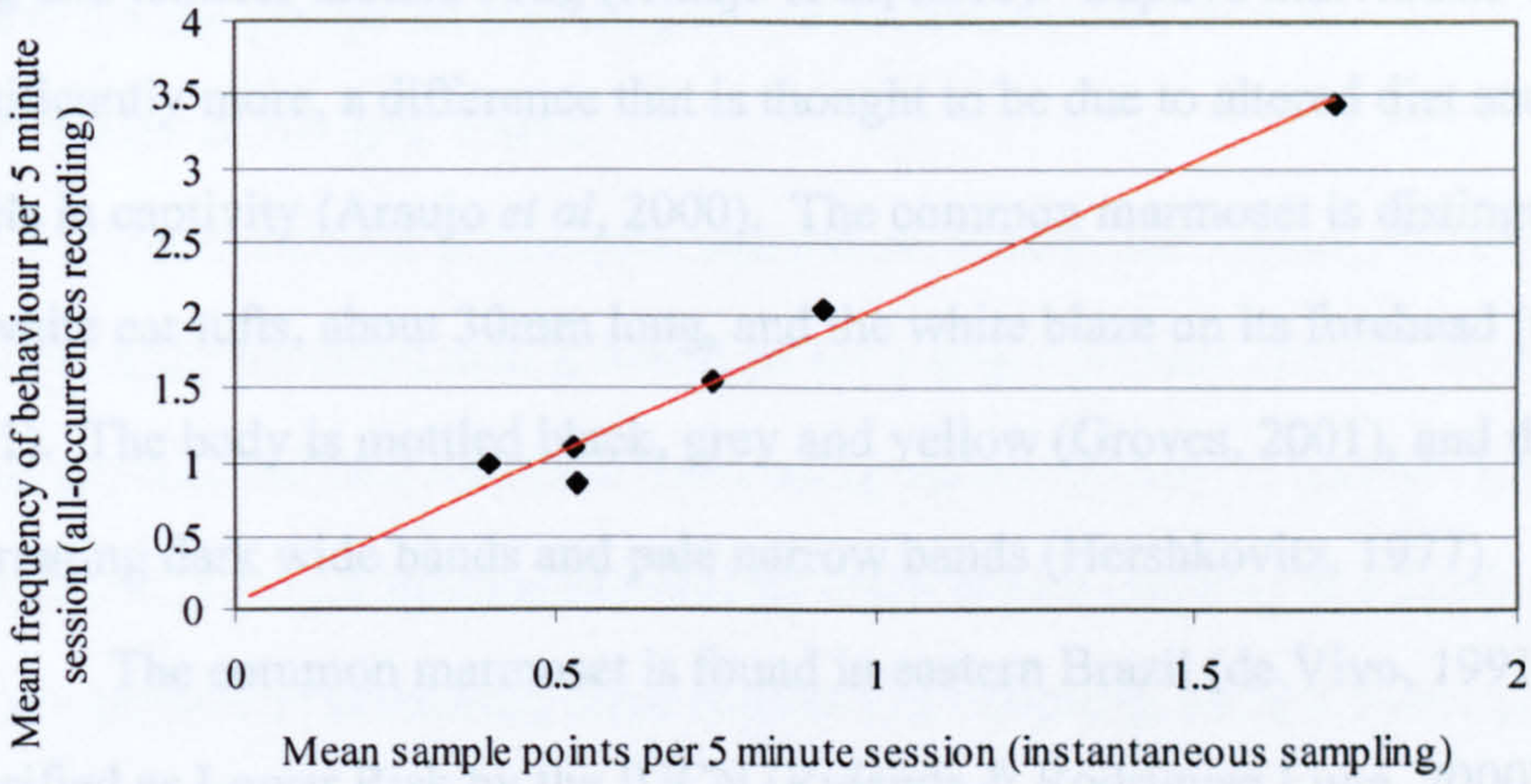
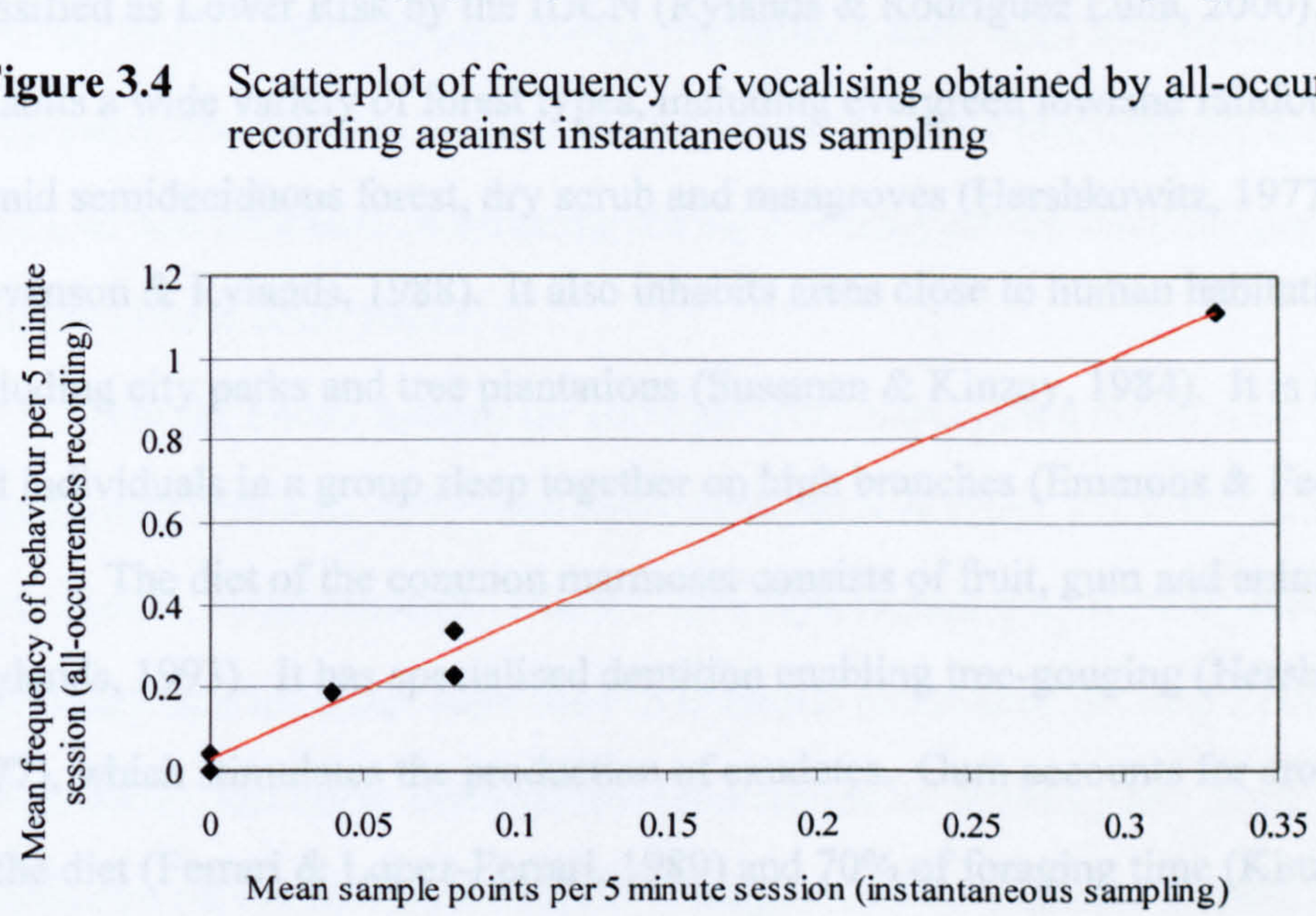


Figure 3.4 Scatterplot of frequency of vocalising obtained by all-occurrences recording against instantaneous sampling



Chapter 4

Identification of behaviours associated with stress and reduced welfare in the common marmoset

(Callithrix jacchus)

4.1 INTRODUCTION

4.1.1 The common marmoset

The common marmoset *(Callithrix jacchus)* (Linnaeus, 1758) is one of the smallest New World primates (Araujo *et al*, 2000). It shows virtually no sexual dimorphism (Hearn, 1983; Hershkovitz, 1977), with wild males weighing around 318g and females around 322g (Araujo *et al*, 2000). Captive individuals weigh significantly more, a difference that is thought to be due to altered diet and activity levels in captivity (Araujo *et al*, 2000). The common marmoset is distinguished by its white ear tufts, about 30mm long, and the white blaze on its forehead (Groves, 2001). The body is mottled black, grey and yellow (Groves, 2001), and the tail has alternating dark wide bands and pale narrow bands (Hershkovitz, 1977).

The common marmoset is found in eastern Brazil (de Vivo, 1991), and is classified as Lower Risk by the IUCN (Rylands & Rodríguez Luna, 2000). It inhabits a wide variety of forest types, including evergreen lowland rainforest, humid semideciduous forest, dry scrub and mangroves (Hershkowitz, 1977; Stevenson & Rylands, 1988). It also inhabits areas close to human habitation, including city parks and tree plantations (Sussman & Kinzey, 1984). It is arboreal, and individuals in a group sleep together on high branches (Emmons & Feer, 1990).

The diet of the common marmoset consists of fruit, gum and animal prey (Rylands, 1993). It has specialised dentition enabling tree-gouging (Hershkowitz, 1977), which stimulates the production of exudates. Gum accounts for around 15% of the diet (Ferrari & Lopez-Ferrari, 1989) and 70% of foraging time (Kinzey *et al*,

1975). Gum represents a spatially clumped and temporally continuous food resource, which enables the home range of this species to be relatively small (0.5-6.5 hectares) (Ferrari & Lopez-Ferrari, 1989).

Studies on wild populations show that common marmosets are diurnal, with peaks in activity in the early morning and late evening, whereas the middle of the day tends to be spent grooming and sleeping (Koening, 1995). Mean group size in the wild is 8.9 (Ferrari & Lopez-Ferrari, 1989) but may be up to 20 individuals (Koening, 1995). The social structure of groups is variable, but tends to consist of multiple males and females, with only the dominant female breeding (Ferrari & Lopez-Ferrari, 1989; Rylands, 1993). Groups with multiple breeding females have also been observed (Digby & Ferrari, 1994; Scanlon *et al*, 1988), although in captive family groups most or all post-pubertal daughters do not ovulate (Abbott, 1984; Evans & Hodges, 1984).

The small size of common marmosets means that reasonable housing space is more easily provided than it would be for a larger primate species, especially for social groups, and also results in them being relatively easy to handle (Johnson *et al*, 1996; Scott, 1991). Common marmosets reach sexual maturity at a fairly young age, and are capable of producing large numbers of offspring, usually twins (Arruda *et al*, 1986; Bruhin, 1979; Epple, 1970a). Marmosets have been bred in captivity for many years; for example, as early as 1937 it was reported that a female common marmoset had produced 24 offspring during a seven-year reproductive period in captivity (Lucas *et al*, 1937).

For the above reasons the common marmoset is used extensively in behavioural (Williams, 1987) and biomedical (Burt & Plant, 1983; Hearn *et al*, 1978; Stellar, 1960) research. Home Office figures state that in 2000, 1060 'marmosets and tamarins' were used in scientific procedures in Great Britain (Home Office, 2001), and many more exist in laboratories for breeding and stock purposes. Results of a survey by EUPREN (1997) suggest that the majority of these were

marmosets (Boyd Group, 2002). Common marmosets have long been used in laboratory studies. For example, Stellar (1960) began a study in 1953 attempting to adapt the marmoset to the laboratory as well as investigating its behaviour. He concluded that common marmosets 'make excellent subjects for behavioral investigations' (Stellar, 1960, p. 1). Until the early 1970's, large numbers of wild marmosets enabled a plentiful supply to be imported for research purposes (Ogden *et al*, 1978). However, embargoes on exportation from source countries resulted in the establishment of breeding colonies (Ogden *et al*, 1978) and today wild-caught marmosets are not available in the United Kingdom (Boyd Group, 2002).

4.1.2 Measures of welfare in the common marmoset

Despite the fact that common marmosets are so widely used in research, there has been a paucity of studies attempting to identify behaviours associated with increased or decreased welfare, resulting from the captive environment, husbandry procedures or experimental manipulations. Johnson and co-workers (1996) found an increase in plasma cortisol in this species, in response to isolation, to be associated with increased movement, which was interpreted as an indicator of behavioural arousal. Increases in plasma cortisol as a result of housing in an unstable peer group, in contrast, were associated with increases in aggressive and submissive behaviours related to agonistic encounters.

Self-scratching is thought to be an indicator of anxiety in many Old World primate species (Maestripieri *et al*, 1992). Anxiety-reducing benzodiazepine drugs have been shown to reduce the frequency of self-scratching in the common marmoset (Cilia & Piper, 1997), suggesting that this may also be true for this New World monkey. In the same study, the anxiolytic drugs resulted in decreases in scent-marking and aggressive behaviour. Increases in allogrooming were also seen following administration of the drug, indicating that muscle relaxation was not responsible for the decreases in the other behaviours seen. However, locomotion

was not affected, suggesting, in contrast to the study by Johnson and co-workers, that this behaviour is not affected by anxiety.

In conclusion, increases in scratching, scent-marking and agonistic behaviours may be indicators of anxiety in this species, although additional validation of each would be useful. Decreases in allogrooming may also be associated with increased anxiety in this species, in contrast to other primate species amongst which it may increase during periods of anxiety, functioning as a tension reduction mechanism (Nieuwenhuijsen & de Waal, 1992; Boccia *et al*, 1989; Schino *et al*, 1988). Evidence is mixed regarding locomotion; it may increase or be unaffected by heightened anxiety. Further data are therefore necessary in order to recognise or repudiate this behaviour as an index of anxiety.

4.1.3 Aim of the present study

The aim of the present study was to validate the use of both behaviour and urinary cortisol as reliable and sensitive measures of stress and therefore welfare in the common marmoset. Both behavioural and physiological measures may be useful as welfare indicators, as was discussed in Chapter 1. A demonstrable positive or negative correlation between urinary cortisol and frequency of a particular behaviour following a stressor will increase the validity of the use of such changes in frequency of the behaviour as an effective indirect welfare indicator. Ewbank (1985) states that four criteria must be fulfilled in order to show a clear relationship between stress and behaviour. These are as follows:

1. The stressor(s) must be identified and (ideally) quantified.
2. The physiological responses (e.g. epinephrine and/or corticosteroid hormone levels) must be quantified and (ideally) correlated with the stressor level and the degree of behavioural change.
3. The behavioural changes must be obvious, fully described, and measured
4. Damage to the physical and/or psychological well-being of the animal must be demonstrated.

(Ewbank, 1985; p.76).

Further to the main aim, there were three goals of this study. The first was to investigate whether exposure to a stressor would produce measurable behavioural changes in this species. The second aim of the study was to establish a baseline for levels of cortisol excreted throughout the day, and to examine whether urinary cortisol production would fluctuate due to diurnal variation, as has been shown for faecal cortisol in this species (Raminelli *et al*, 2001; Sousa & Ziegler, 1998) and urinary cortisol in Weid's black tufted-ear marmosets (Smith & French, 1997). Finally, it was intended to examine whether exposing marmosets to a stressor would result in reliable increases in urinary cortisol, and to assess the time period between exposure to the stressor and the resulting elevation in cortisol levels. If significant effects were found, an attempt would be made to correlate these with changes in behaviour. This would add validity to the use of such behavioural measures as a relatively simple method of assessing welfare in this species.

4.2 METHODS

4.2.1. Study animals and housing

The study animals were 24 common marmosets, 12 males and 12 females, with a mean age of 1089 days (± S.E. 135.67) as of 01/02/01. Animals in Group 1 (n=12 animals) had a mean age of 1188 days (± S.E. 232.37 days) and those in Group 2 (n=12 animals) a mean age of 989 days (± S.E. 145.55 days). The ages of animals in the two groups were not significantly different to each other (t_{12}=0.72; p=0.13). The animals were housed in male-female pairs at the Medical Research Council (MRC) Human Reproductive Sciences Unit, Bush Estate, Edinburgh. See Chapter 3 for details of housing and husbandry routines. None of the females used in the study was past the first trimester of pregnancy, as determined by transabdominal uterine palpations, which were performed regularly. This is generally considered to be a reliable method for detecting pregnancy in this species

(Hearn *et al*, 1978). This was important as cortisol levels may be affected by pregnancy (Bazer, 1998).

4.2.2 Experimental Design

Study animals were assigned to one of two test groups. All animals in Group 1 (trained animals) were housed in the same colony room (Room 6), and those in Group 2 (untrained animals) in an adjacent colony room (Room 5).

Group 1 (trained) animals

Training procedure

Animals in Group 1 were trained by Jean McKinley over a period of approximately six weeks to provide urine samples for analysis. Scent marking is a behaviour that occurs fairly frequently in common marmosets in general (Epple, 1970b; Epple *et al*, 1993; Lazaro-Perea *et al*, 1999) and in this population of animals in particular. When scent marking occurs, a few drops of urine are usually deposited on the substrate (Epple, 1970b), which in the case of the study population was generally one of the branches within the cage.

During training, animals were initially rewarded for scent marking with a prized food item (a small piece of marshmallow). When the animals had begun to scent mark as soon as the trainer had sat in front of the cage, a verbal request was added. The request was initially made as the animals were moving towards an area on a branch to scent mark, and later when they were elsewhere in the cage. When the marmosets had begun to scent mark on request, the trainer noted the location that each animal seemed to use most frequently, and it was from then on only rewarded for scent marking in this location. The branches in the cage were removed and holes drilled in the scent marking location, which was generally easy to identify, as the branch was wet in this area. Collecting vials could then be inserted directly into the branch at the locations preferred by the animals (see Plates 11 and

Plate 11 Vial used for urine collection

Plate 12 Vial inserted into hole drilled in branch

12). Urine was then collected, in combination with behavioural data, in two stages. Initially, data were collected throughout the day to examine the effects of circadian rhythms on urinary cortisol and behaviour. Secondly, the same parameters were studied following administration of an acute stressor.

Response to a stressor

On the day of stressor administration, each of the animals was chased into the nest-box, which was then closed and removed from the cage. The nest-box was then taken into a separate room and the marmosets removed one at a time and transferred by gloved hand to a small cage to be weighed. They were then returned to the nest-box, which was then replaced in the home cage, and opened to allow the animals to re-enter the home cage at will. The whole procedure took between 4 min and 4 min 30 seconds (mean time 4 min 9 sec; ± S.E. 4.73 sec), and was carried out on 07/03/01 (a Wednesday) between 0930h and 1030h. Removal from the home cage for weighing is a routine laboratory procedure and is carried out several times a year.

Group 2 (untrained) animals

A consequence of the urine training carried out with animals from Group 1 was that they became extremely habituated to the presence of humans, and they appeared to become less fearful when exposed to laboratory procedures. To examine whether this was the case matched behavioural data were collected from animals in an adjacent colony room. Behavioural data were collected at 1000h, 1200h, 1400h and 1600h on one day, and the stressors were administered between 0930h and 1000h the following day. The stressors were identical to those used for Group 1 animals and these animals were removed from the home cage for between 3 min 45 seconds and 4 min 30 seconds (mean time 4 min 14 sec; ± S.E. 7.24 sec).

The amount of time spent away from the home cage was not significantly different for animals in Group 1 and Group 2 (t_{10}=-0.578; p=0.58).

Cortisol enzyme immunoassay (EIA)

Dr. Tessa Smith of Queen's University, Belfast, measured cortisol concentrations in all urine samples. The enzime immunoassay was immunologically validated as described by Reimers and colleagues (1996). Serial dilutions of four urine pools gave parallel displacement curves with a standard solution. This confirmed that the cortisol in the urine samples was immunologically identical with standard cortisol preparations (from Sigma Chemical Company). Recovery of known amounts of cortisol standard (n = 5 stds: 500, 250, 125, 62.5, 31.25 pg/50ul) from high and low concentrations of a urine pool had a mean of 80.83 ± S.E. 1.9 (n = 3 repeats for high pool and 3 repeats for low pool). Intra-assay coefficients of variation for high and low concentration pools were 4.68 and 1.91% respectively (n=11). Inter-assay coefficients of variation for high and low concentration pools were 9.30 and 14.89% respectively (n=11). Sensitivity was 1.95 pg/50ul, equivalent to 39 pg/1ml. To correct for urine dilution, creatinine concentrations were quantified for each sample (Tietz, 1976) and cortisol expressed as μg cortisol/mg Cr/ml.

4.2.3 Data collection

Behavioural data were recorded just prior to the collection of urine. Instantaneous scan sampling was used with an interval of 15 seconds between scans; data collection sessions lasted for 5 minutes. Data were collected on a palm top computer using THE OBSERVER 3.0 software. Behaviours recorded included: inactive, alert and watching the observer (henceforth referred to as 'watch observer'); inactive, alert and not watching the observer (referred to as 'inactive, alert'); 'locomote'; 'self-scratch'; 'scent mark'; 'vocalise' and 'forage' (see Chapter

3, page 89, for behavioural definitions). The two categories of inactive behaviour were also collapsed for the purposes of the analysis to form 'inactive (both categories)'. These behaviours were selected because they were easily distinguished and relatively frequent, and some of them have been proposed as welfare indicators. An 'other' category was also used, and included behaviours very infrequently seen, such as allogrooming and inactive, inalert behaviour. Additionally, for each scan, it was recorded whether the animal was in or out of the nest-box.

For trained animals, in order to examine whether circadian rhythms were associated with fluctuations in behaviour and levels of urinary cortisol throughout the day, behavioural data and samples of urine were collected from each animal every hour from 0900h – 1600h. A total of at least eight urine samples per animal and eight behavioural samples per pair were therefore collected. This was during the light phase of the 12:12 light/dark cycle, which started at 0800h and ended at 2000h. The collection of these baseline data took place over a two-week period during early February 2001. Urine samples were collected over a range of n=3-7 days, and behavioural data over a range of n=4-8 days. In the event that more than one urine sample was obtained per animal for any single hour, a mean was used for the purposes of the analysis.

Table 4.1 Times and days that behavioural data and matching urine samples were collected

Time	Stressor Day	Stressor day +1	Stressor day +2	Stressor day +7
10.00	Stressors administered	✓	✓	✓
12.00	✓			
14.00	✓			
16.00	✓			

On the day that the stressor was administered, behavioural data and urine were collected from trained animals at 1200h, 1400h and 1600h, and at 1000h on three further days subsequent to this (see Table 4.1). Animals were not subject to

any further stressful procedures on any of the seven days following administration of the stressor. For the untrained animals, behavioural data were recorded after stressors had been administered (using the same behavioural categories as were used for Group 1) at 1000h, 1200h, 1400h and 1600h.

4.2.4 Statistical analysis

Behavioural data

Trained animals

1. Circadian rhythms

Single-factor repeated-measures ANOVAs were carried out on data collected every hour from 0900 to 1600h to see whether any (pre-stressor) behaviours were significantly affected by time of day. Additionally, mean behavioural frequencies for two-hour blocks (0900-1000; 1100-1200; 1300-1400 and 1500-1600h) were calculated, and further repeated-measures ANOVAs carried out on these values.

2. Response to a stressor

A two-factor repeated-measures analysis of variance was carried out to determine whether behaviour changed during the hours following the stressor (on the day that the stressor was administered). The factors analysed were 'stress' and 'time period' (see Table 4.2), as well as the interaction between the two.

Table 4.2 Variables analysed to examine 'response to a stressor' for trained animals, and number of levels within each

Variable	Within / between subjects	No. of levels	Levels
Stress	Within-subjects	2	Pre-stressor Post-stressor
Time period	Within-subjects	3	1200h 1400h 1600h

3. Effect of day

Another single factor repeated-measures ANOVA was carried out to look at effect of day (in relation to stressor) on behaviour. Data collected at 1000h on four different days (pre-stressor), one day after the stressor (stressor +1), two days after the stressor (stressor +2) and seven days after the stressor (stressor +7) were compared.

Untrained animals

Response to a stressor

a) Time periods 1000h, 1200h, 1400h and 1600h

A two-factor repeated-measures analysis of variance was carried out to determine whether behaviour changed following the stressor. The factors analysed were 'stress' and 'time period' (see Table 4.3), and the interaction between the two.

Table 4.3 Variables analysed to examine 'response to a stressor' for untrained animals, and number of levels within each

Variable	Within / between subjects	No. of levels	Levels
Stress	Within-subjects	2	Pre-stressor Post-stressor
Time period	Within-subjects	4	1000h 1200h 1400h 1600h

In contrast to the analysis carried out on data from trained animals, here it was possible to use four time periods as behavioural data had been collected at 1000h on the stressor day.

b) Time periods 1200h, 1400h and 1600h

In order to eliminate the possibility that any differences seen in the results of the analyses between trained and untrained animals were due to the inclusion of 1000h data in the untrained animal analysis, another repeated-measures ANOVA was carried out, using only time periods 1200h, 1400h and 1600h. Variables analysed in this ANOVA were the same as those for the previous analysis.

Trained and untrained animals combined

A three-factor mixed analysis of variance was carried out using data from both trained and untrained animals. This was to see if there was an effect of training on behaviour, and also to effectively increase the sample size by combining both sets of data. The variables analysed were 'stress', 'time period' and 'training' (see Table 4.4). The interactions between 'stress' and 'time period', 'stress' and 'training' and 'time period' and 'training' were also analysed, as was the three-way interaction between all the variables.

Table 4.4 Variables analysed to examine 'response to a stressor' for trained and untrained animals, and number of levels within each

Variable	Within / between subjects	No. of levels	Levels
Stress	Within-subjects	2	Pre-stressor Post-stressor
Time period	Within-subjects	3	1200h 1400h 1600h
Training	Between-subjects	2	Group 1 (trained) Group 2 (untrained)

Significance was set at alpha <0.05 throughout the analyses. Where significant main effects were found using repeated-measures ANOVAs, where

appropriate, post-hoc pairwise *t*-tests with the Bonferroni correction were used. These were intended to pinpoint where differences lay, while controlling against Type I errors. The behaviour of each animal was likely to have been influenced by that of its cage mate, and so data from each individual could not be treated as independent. For this reason, a single mean was calculated from the data of both animals; each pair was effectively treated as one individual in the analysis. Data used consisted of mean sample points per session; 20 sample points were obtained per pair per 5 minute session. Data were found to be normally distributed throughout and hence parametric tests were used.

Statistical analysis of cortisol data

1. Circadian rhythms

Concentrations of urinary cortisol were assessed for each animal at each of eight time periods (0900, 1000, 1100, 1200, 1300, 1400, 1500, 1600h) and data analysed using a mixed 2-factor ANOVA. Sex of animal was a between-subjects factor (male vs. female) and time was a within-subjects factor. Mean cortisol values were also analysed for each sex separately, using a repeated-measures ANOVA in each case.

In addition, mean cortisol values for two hour time blocks were calculated for each animal and analysed again using a mixed 2-factor ANOVA. The four time blocks were 0900-1000, 1100-1200, 1300-1400 and 1500-1600h. Again, repeated-measures ANOVAs were used to test for effect of time on each sex separately.

2. Response to a stressor

A two-factor within-subjects ANOVA was used to test for effect of stress condition (pre- and post-stressor) and time of day (1200, 1400 and 1600h). Stressors were administered at around 1000h, and it is known that cortisol takes some time to show in the urine; therefore in a second analysis, baseline 1000h and 1000h post-

stressor values from the morning after the stressor day were included. Cortisol concentrations in urine samples taken before the stressor and 1, 2, 5 and 7 days after the stressor were also compared using a repeated-measures ANOVA.

4.3 RESULTS

4.3.1 Behavioural analyses

The results are structured so that all main effects are initially presented. Any significant interactions are presented later.

1. Circadian rhythms (Trained animals only)

No behaviours were significantly affected by time of day (see Table 4.5) when data were analysed in the original 1-hour time periods. When data were collapsed into 2-hour time blocks for each behaviour, 'inactive, alert' was significantly affected by time of day (see Table 4.6).

Table 4.5 Results of within-subjects ANOVAs of effects of time of day (data in 1-hour time blocks) on all behaviours (Pre-stressor; trained animals only)

Behaviour	F	p
Watch observer	0.54	0.80
Inactive, alert	2.18	0.06
Inactive (both categories)	0.09	0.99
Locomote	0.66	0.70
Forage	0.74	0.64
Self-scratch	0.94	0.49
Scent mark	0.87	0.54
Vocalise	1.33	0.26
In nest box	1.54	0.19

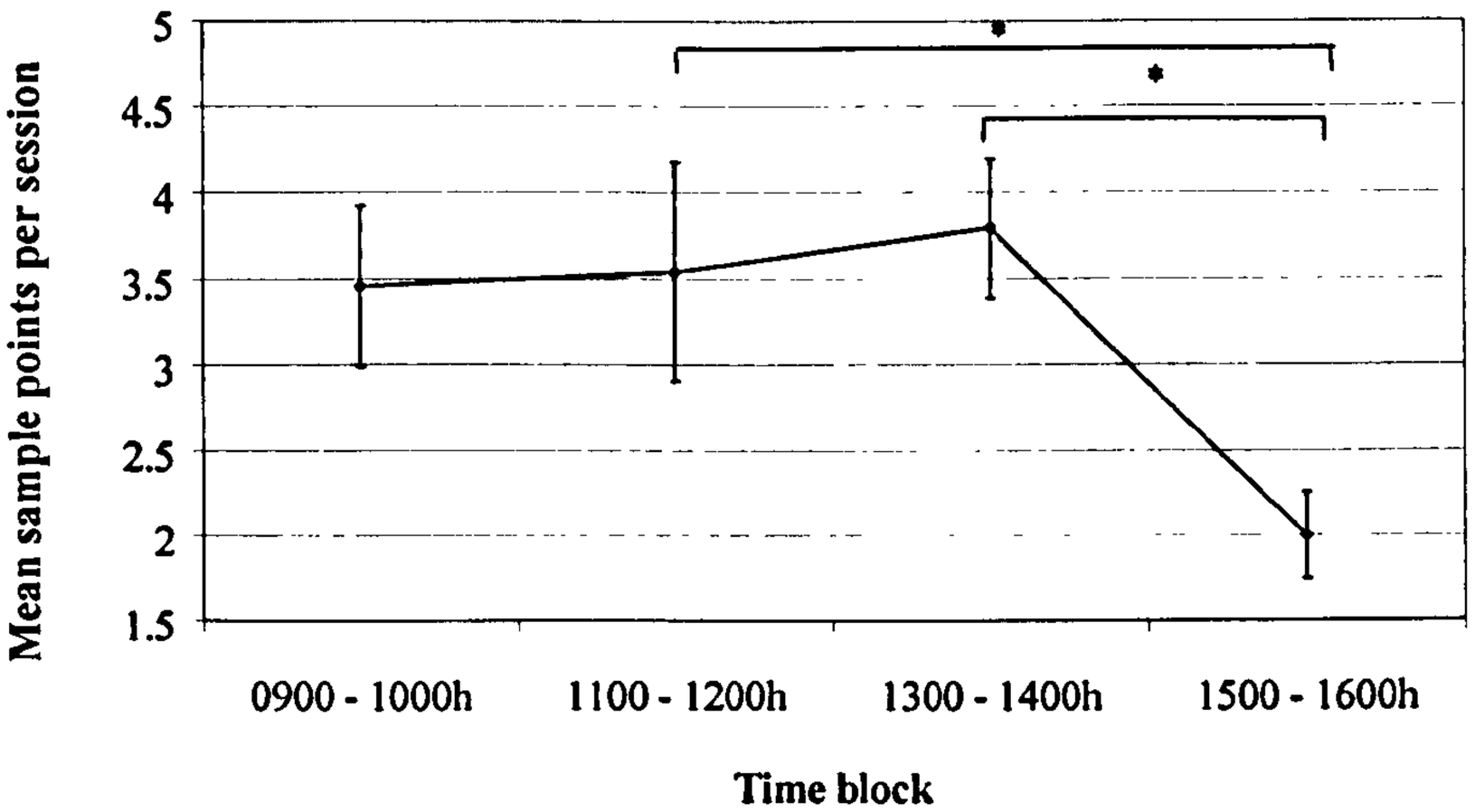
All d.f. = 7,35

Table 4.6 Results of within-subjects ANOVAs of effects of time of day (data collapsed into 2-hour time blocks) on all behaviours (Pre-stressor; trained animals only)

Behaviour	F	p
Watch observer	2.71	0.08
Inactive, alert	5.48	<0.01**
Inactive (both categories)	0.21	0.89
Locomote	1.25	0.33
Forage	0.90	0.47
Self-scratch	0.63	0.61
Scent mark	1.64	0.22
Vocalise	1.60	0.23
In nest box	1.21	0.34

All d.f. = 3,15 * p<0.05

Figure 4.1 Mean sample points spent 'inactive, alert' over time for trained animals (bars represent standard errors) (Pre-stressor; trained animals only)



* p<0.05

'Inactive, alert' was relatively constant over time for the first three time blocks, but decreased for block 4 (1550-1600h) (see Figure 4.1). Post-hoc *t*-tests revealed significant differences in frequencies of this behaviour between 1100-

1200h and 1500-1600h, as well as between 1300-1400h and 1500-1600h (see Table 4.7).

Table 4.7 Post-hoc *t*-test *t* and p values for effects of time (in 2-hour blocks) on 'inactive, alert' behaviour

Time Blocks	***t***	**p** (uncorrected)	**p** (following Bonferroni correction)
0900-1000h vs. 1100-1200h	0.15	0.89	1.00
0900-1000h vs. 1300-1400h	0.56	0.59	1.00
0900-1000h vs. 1500-1600h	2.38	<0.05*	0.22
1100-1200h vs. 1300-1400h	0.74	0.46	1.00
1100-1200h vs. 1500-1600h	2.85	<0.01**	<0.05*
1300-1400h vs. 1500-1600h	3.07	<0.01**	<0.05*

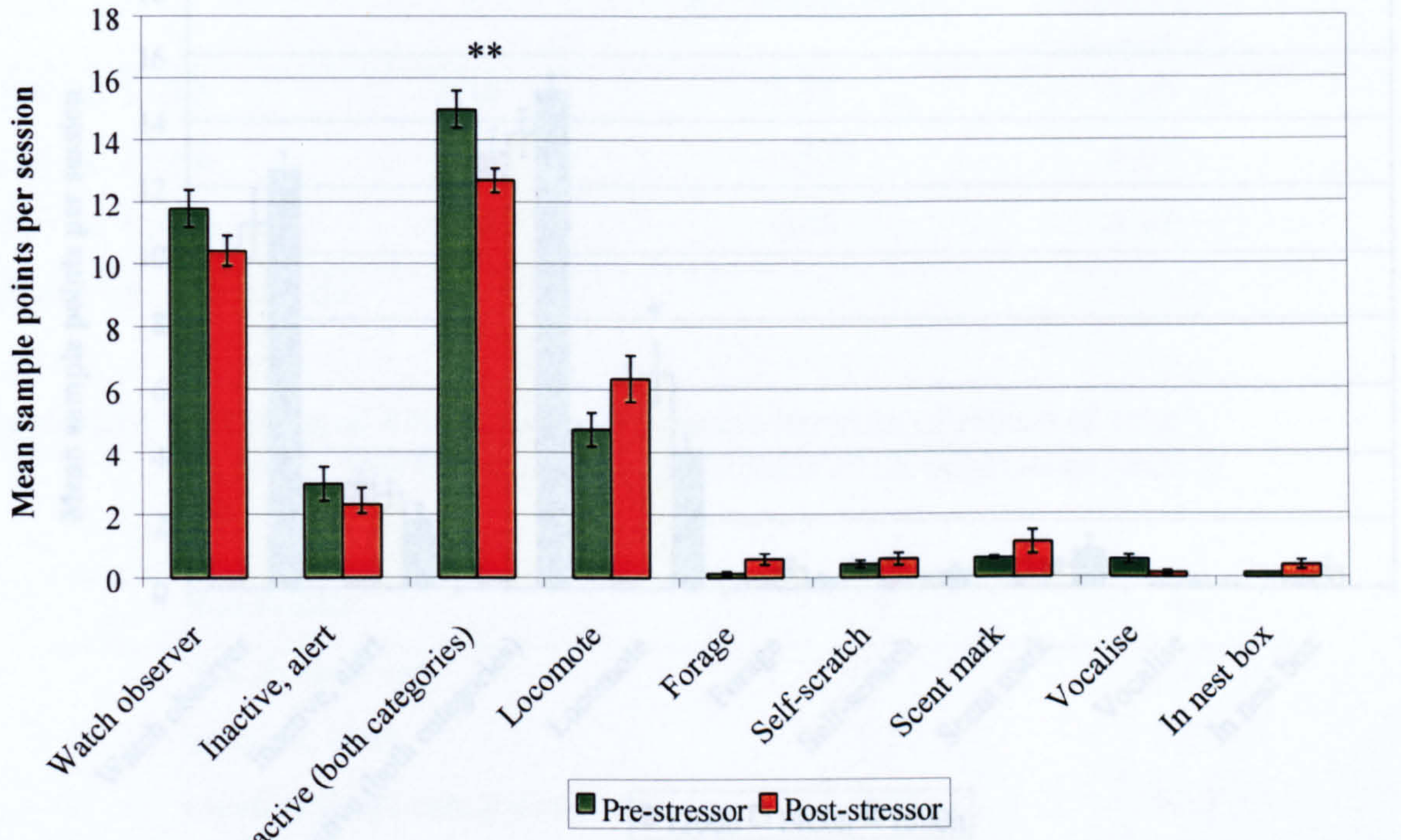
All d.f. = * p<0.05; ** p<0.01

2. Response to stressors

a. Trained animals

There was significantly less 'inactivity (both categories)' after the stressor compared with before it (see Figure 4.2). There were apparent trends indicating effects of the stressor on other behaviours (*e.g.* 'watch observer', 'locomote', 'forage' and 'scent mark') but none of these reached significance (see Figure 4.2 and Table 4.8). There was also an effect of time of observation on 'locomote' (see Figure 4.3 and Table 4.9). However, following the Bonferroni correction, no significant differences between the individual observation times were found (see Table 4.10). There were no significant interactions between the variables of 'stress' and 'time' for any of the behaviours (see Table 4.11).

Figure 4.2 Mean sample points spent performing each behaviour before and after the stressor (trained animals, collapsed across 1200, 1400 and 1600h) (bars represent standard errors)



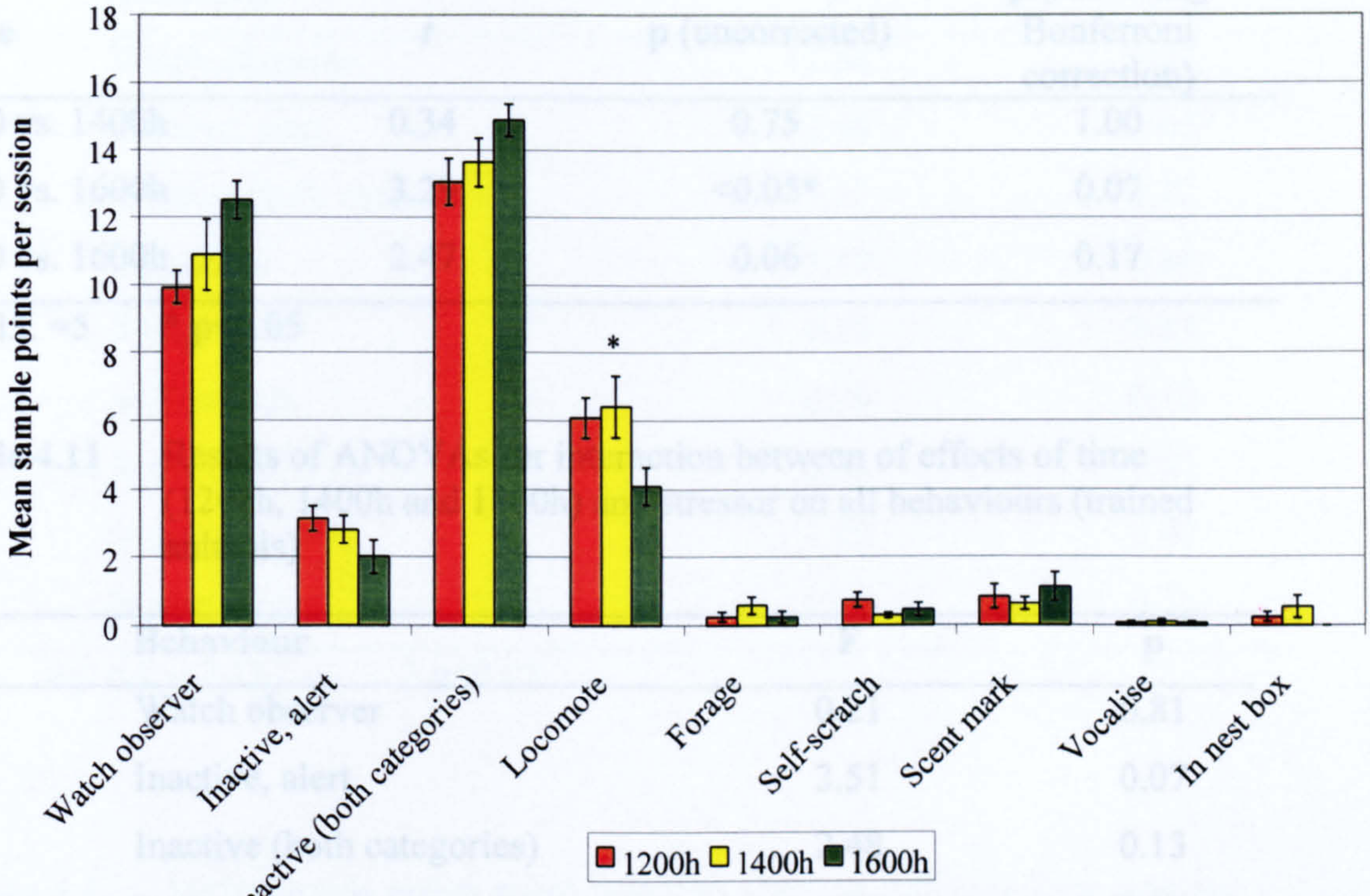
** - p<0.01

Table 4.8 Results of within-subjects ANOVAs of effects of stressor on all behaviours (trained animals, collapsed across 1200, 1400 and 1600h)

Behaviour	F	p
Watch observer	5.43	0.07
Inactive, alert	0.96	0.37
Inactive (both categories)	36.14	<0.01**
Locomote	4.00	0.10
Forage	4.00	0.10
Self-scratch	0.63	0.47
Scent mark	4.22	0.10
Vocalise	0.65	0.46
In nest box	0.68	0.45

All d.f. =1,5

Figure 4.3 Mean sample points spent performing each behaviour at three successive time periods (trained animals, pre- and post-stressor data combined) (bars represent standard errors)



* p<0.05

Table 4.9 Results of within-subjects ANOVAs of effects of time (1200h, 1400h and 1600h) on all behaviours (trained animals, pre- and post-stressor data combined)

Behaviour	F	p
Watch observer	3.26	0.08
Inactive, alert	1.99	0.19
Inactive (both categories)	3.05	0.09
Locomote	4.37	<0.05*
Forage	0.76	0.49
Self-scratch	2.25	0.16
Scent mark	1.34	0.31
Vocalise	0.22	0.81
In nest box	2.36	0.15

All d.f. = 2,10

Table 4.10 Post-hoc *t*-test *t* and p values for effects of time on 'locomote' (trained animals, pre- and post-stressor combined)

Time	*t*	p (uncorrected)	p (following Bonferroni correction)
1200 vs. 1400h	0.34	0.75	1.00
1200 vs. 1600h	3.29	<0.05*	0.07
1400 vs. 1600h	2.47	0.06	0.17

All d.f. =5 * p<0.05

Table 4.11 Results of ANOVAs for interaction between of effects of time (1200h, 1400h and 1600h) and stressor on all behaviours (trained animals)

Behaviour	F	p
Watch observer	0.21	0.81
Inactive, alert	3.51	0.07
Inactive (both categories)	2.48	0.13
Locomote	1.79	0.22
Forage	0.04	0.96
Self-scratch	0.92	0.43
Scent mark	0.54	0.60
Vocalise	0.71	0.51
In nest box	0.66	0.54

All d.f. = 2,10

4. Effect of day

There was a significant effect of day on 'inactive alert', but no effects on any of the other behavioural categories (see Table 4.12 and Figure 4.4). Post-hoc pairwise *t*-tests showed no overall difference between any of the days (see Table 4.13).

Table 4.12 Results of within-subjects ANOVAs of effects of day on all behaviours (trained animals only)

Behaviour	F	P
Watch observer	1.64	0.22
Inactive, alert	3.34	<0.05*
Inactive (both categories)	1.67	0.22
Locomote	3.19	0.05
Forage	0.29	0.83
Self-scratch	2.29	0.12
Scent mark	0.33	0.81
Vocalise	1.00	0.42
In nest box	1.24	0.33

All d.f. = 3,15 * p<0.05

Figure 4.4 Mean sample points spent 'inactive, alert' at 1000h on four separate days (trained animals only) (bars represent standard errors)

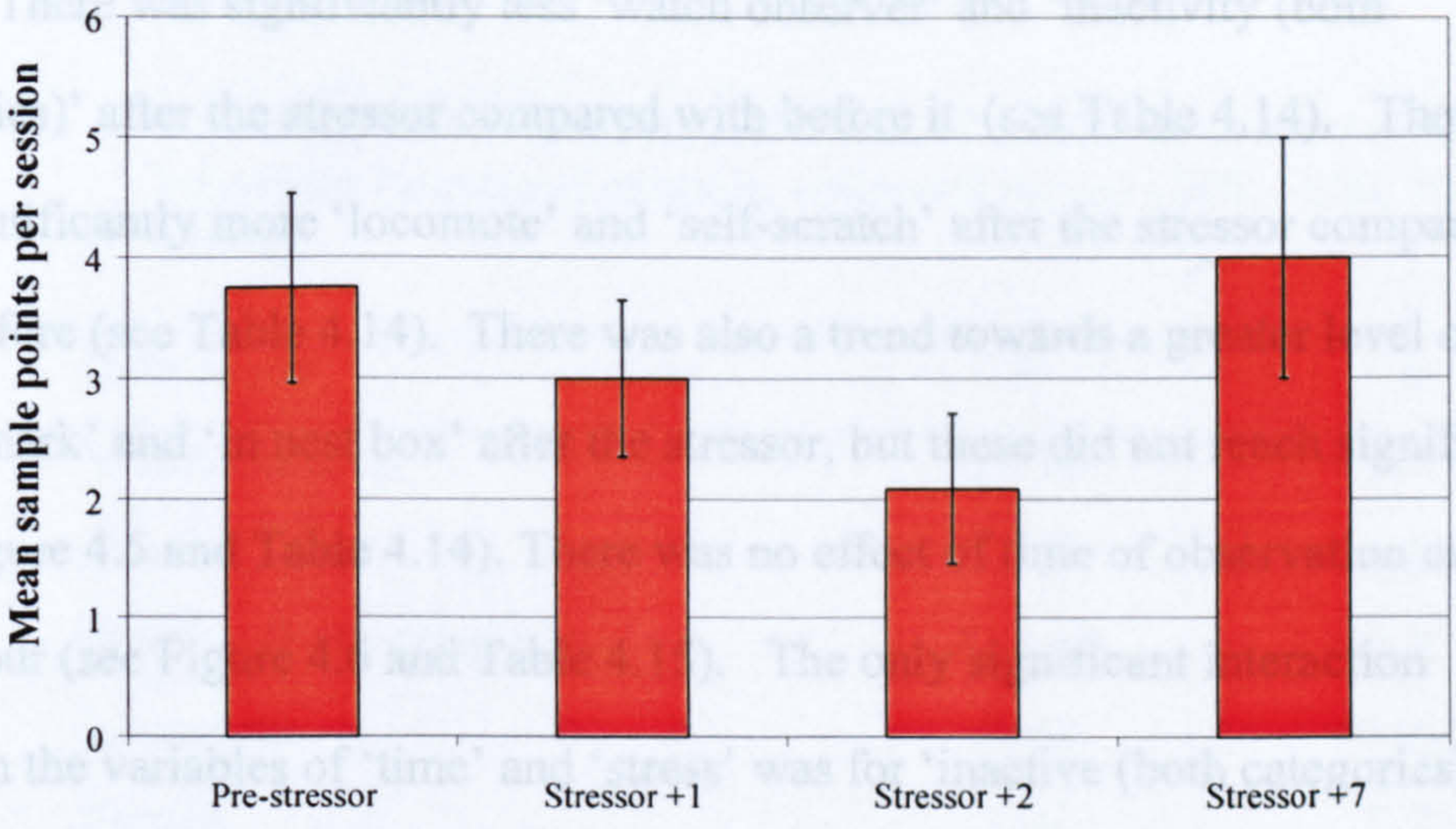


Table 4.13 Post-hoc *t*-test *t* and p values for time spent 'inactive, alert' at 1000h on four separate days (trained animals only)

Day	*t*	p (uncorrected)	p (following Bonferroni correction)
Pre-Stressor vs. Stressor +1	1.10	0.32	1.00
Pre-Stressor vs. Stressor +2	3.11	<0.05*	0.16
Pre-Stressor vs. Stressor +7	0.34	0.75	1.00
Stressor +1 vs. Stressor +2	1.25	0.27	1.00
Stressor +1 vs. Stressor +7	1.88	0.12	0.71
Stressor +2 vs. Stressor +7	2.21	0.08	0.47

All d.f. = 5 * p<0.05

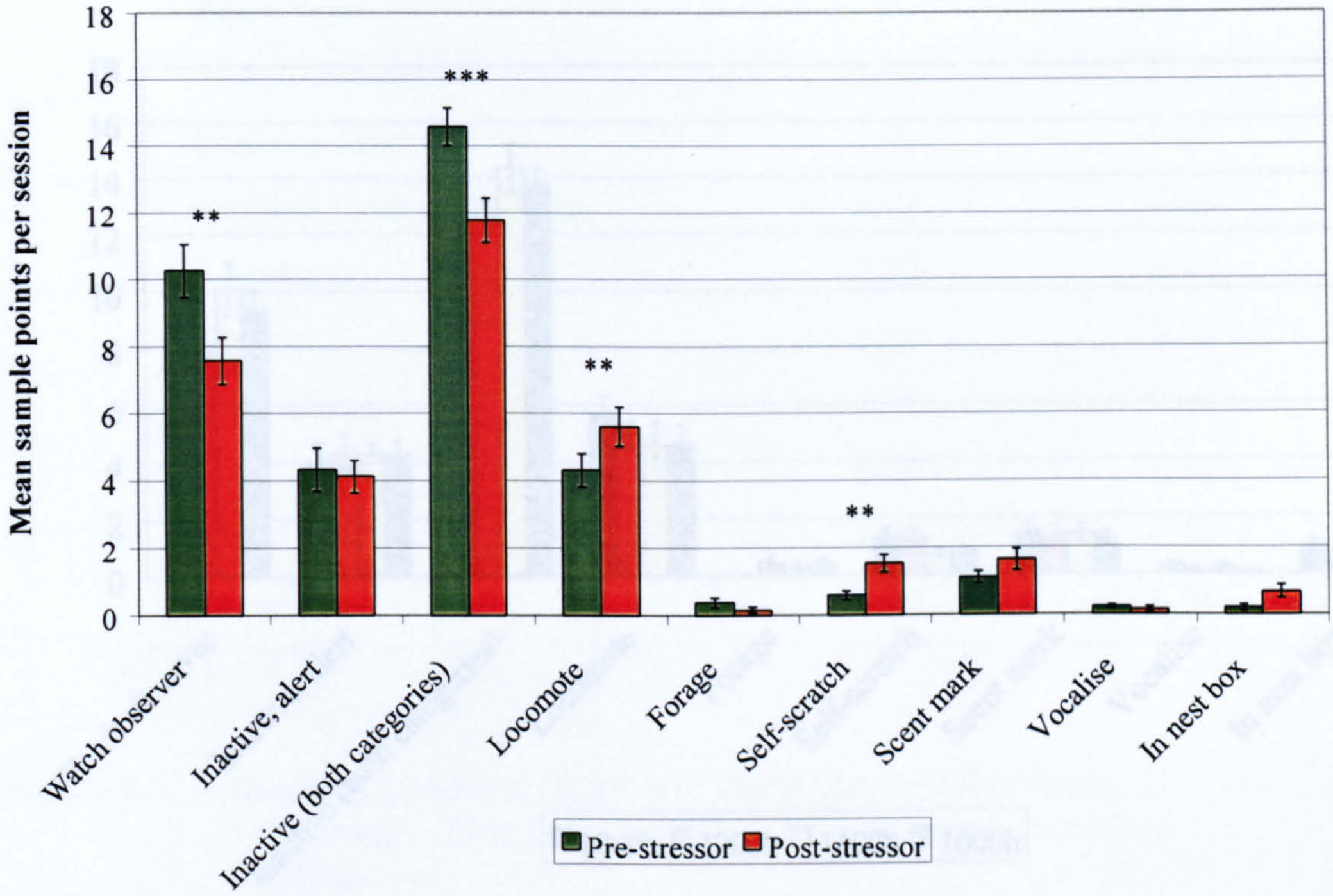
Untrained animals

Response to a stressor

a) Time periods 1000h, 1200h, 1400h and 1600h

There was significantly less 'watch observer' and 'inactivity (both categories)' after the stressor compared with before it (see Table 4.14). There was also significantly more 'locomote' and 'self-scratch' after the stressor compared with before (see Table 4.14). There was also a trend towards a greater level of 'scent mark' and 'in nest box' after the stressor, but these did not reach significance (see Figure 4.5 and Table 4.14). There was no effect of time of observation on behaviour (see Figure 4.6 and Table 4.15). The only significant interaction between the variables of 'time' and 'stress' was for 'inactive (both categories)' (see Figure 4.7 and Table 4.16). Levels of this behaviour remained relatively stable over time for the pre-stressor condition, but after the stressor they were dramatically reduced at 1000h. At 1200h, post-stressor levels had risen slightly. They rose again at 1400h, and rates were similar pre- and post-stressor at 1600h.

Figure 4.5 Mean sample points spent performing each behaviour before and after the stressor (untrained animals, collapsed across 1000, 1200, 1400 and 1600h) (bars represent standard errors)



**p<0.01; ***p<0.001

Table 4.14 Results of within-subjects ANOVAs of effects of stressor on behaviour (untrained animals, collapsed across 1000, 1200, 1400 and 1600h)

Behaviour	F	p
Watch observer	29.16	<0.01**
Inactive, alert	0.38	0.57
Inactive (both categories)	117.28	<0.001***
Locomote	60.06	<0.01**
Forage	2.07	0.21
Self-scratch	50.37	<0.01**
Scent mark	2.42	0.18
Vocalise	2.50	0.18
In nest box	4.05	0.10

All d.f. = 1,5

Figure 4.6 Mean sample points spent performing each behaviour at four successive time periods (untrained animals, pre- and post- stressor data combined) (bars represent standard errors)

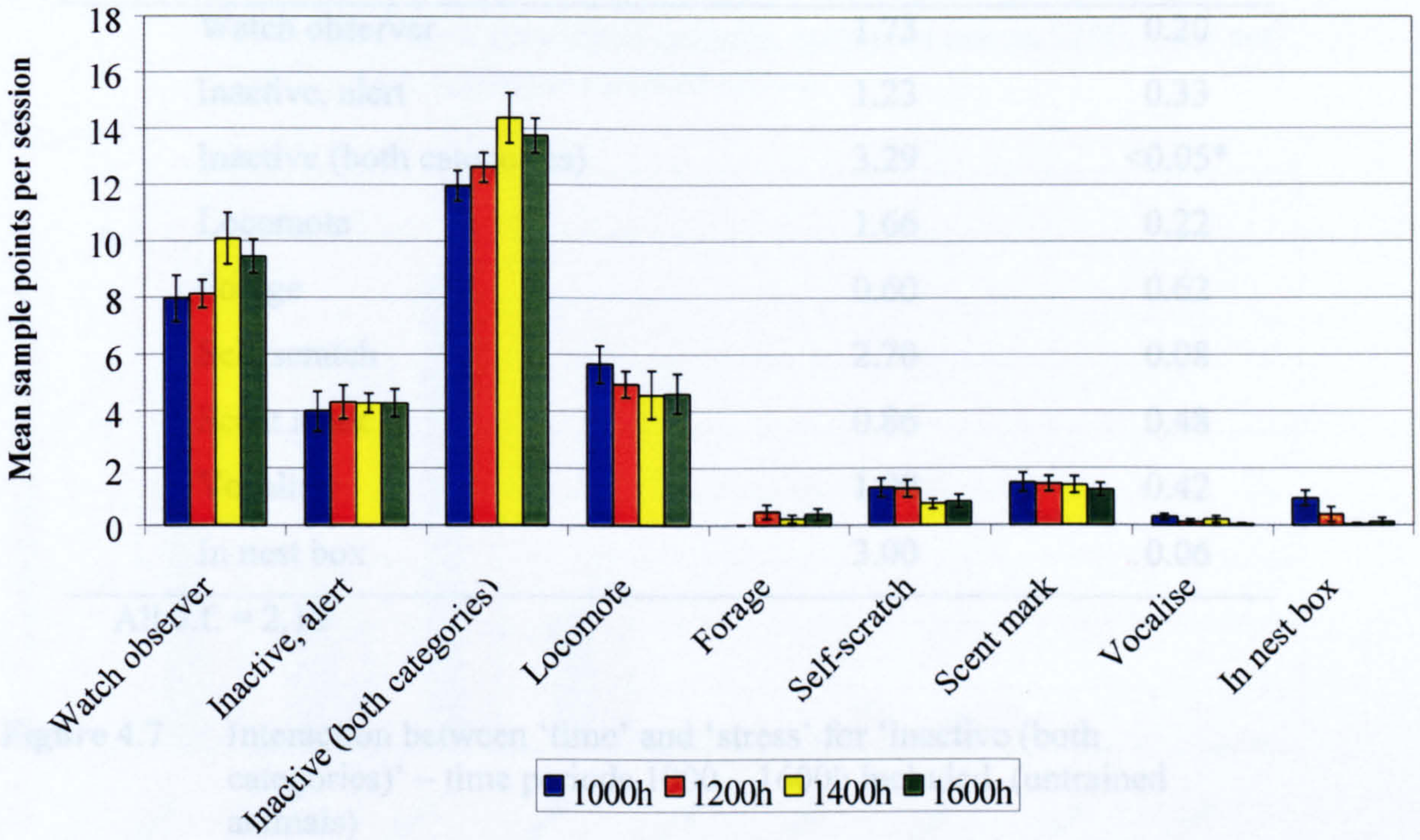


Table 4.15 Results of within-subjects ANOVAs of effects of time (1000h,1200h, 1400h and 1600h) on all behaviours (untrained animals, pre- and post-stressor combined)

Behaviour	F	p
Watch observer	2.62	0.09
Inactive, alert	0.11	0.95
Inactive (both categories)	2.91	0.07
Locomote	1.01	0.42
Forage	1.34	0.30
Self-scratch	1.10	0.38
Scent mark	0.48	0.70
Vocalise	1.32	0.31
In nest box	2.71	0.08

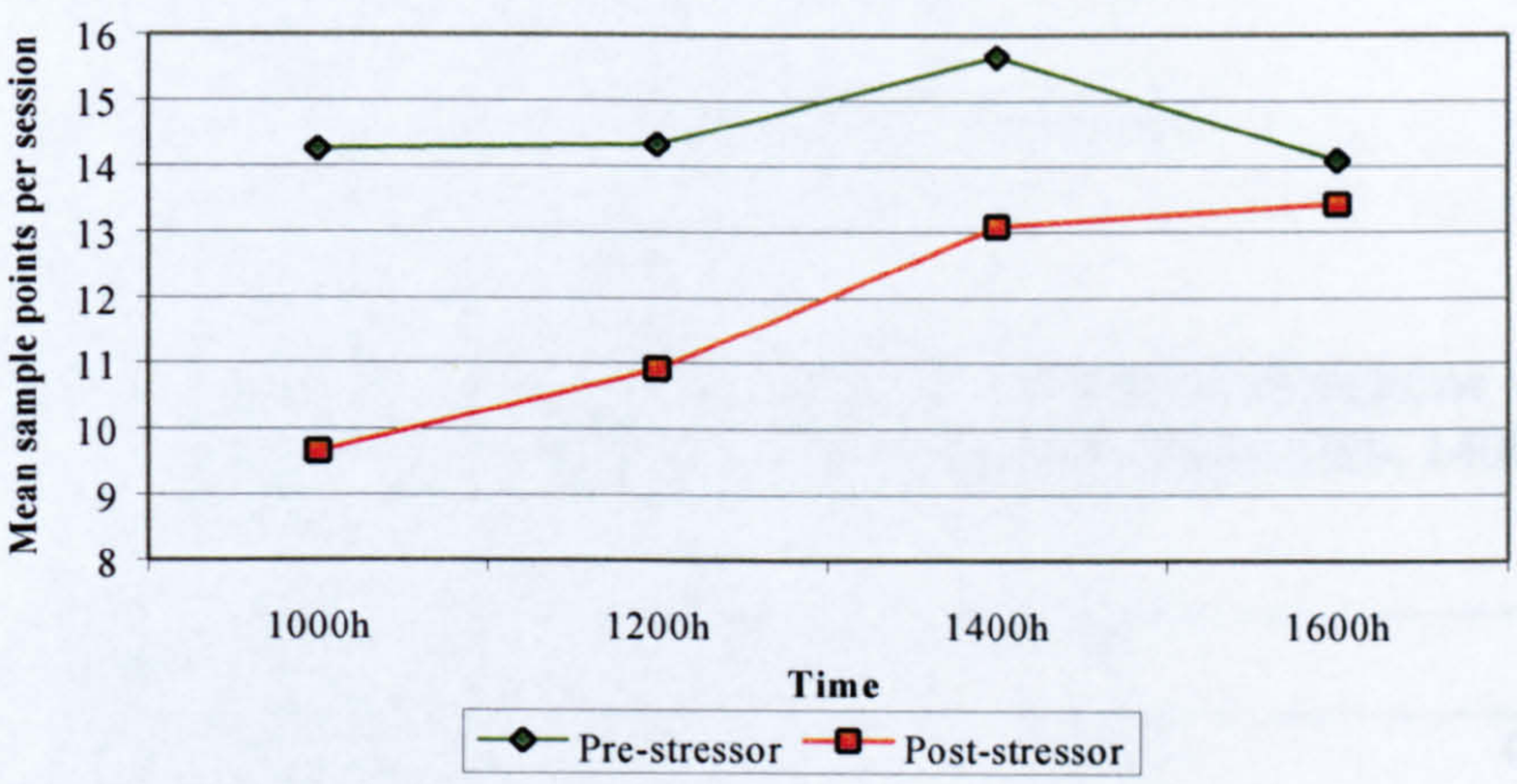
All d.f. = 3,15

Table 4.16 Results of ANOVAs for interaction between of effects of time (1000h, 1200h, 1400h and 1600h) and stressor on all behaviours (untrained animals)

Behaviour	F	p
Watch observer	1.73	0.20
Inactive, alert	1.23	0.33
Inactive (both categories)	3.29	<0.05*
Locomote	1.66	0.22
Forage	0.60	0.62
Self-scratch	2.70	0.08
Scent mark	0.86	0.48
Vocalise	1.00	0.42
In nest box	3.00	0.06

All d.f. = 2,10

Figure 4.7 Interaction between 'time' and 'stress' for 'inactive (both categories)' – time periods 1000 – 1600h included (untrained animals)

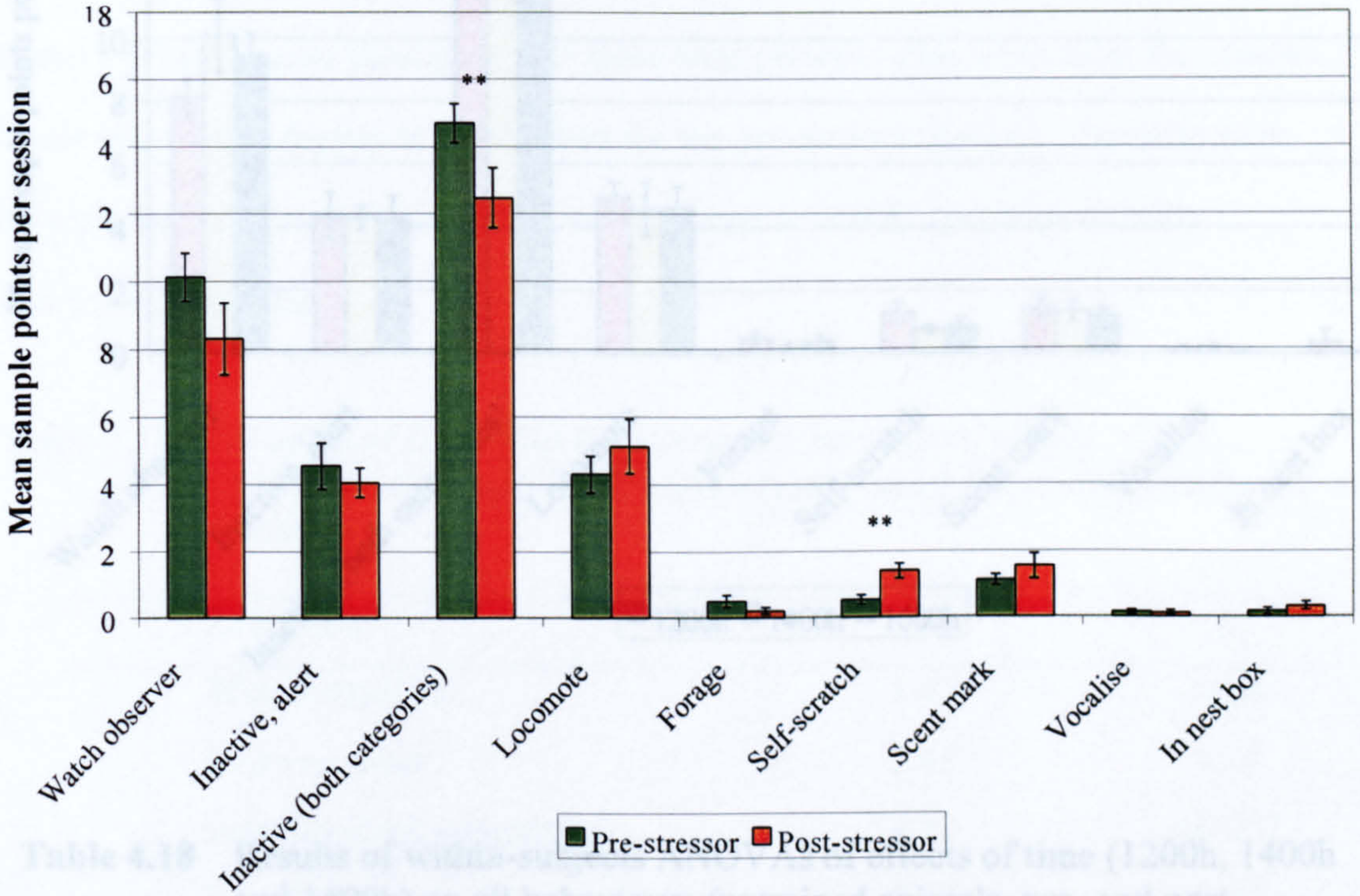


b) Time periods 1200h, 1400h and 1600h

There was significantly less 'inactivity (both categories)' after the stressor compared with before it (see Figure 4.8). There was significantly more 'self-scratch' after the stressor compared with before it (see Figure 4.8). There were also trends towards greater levels of 'locomote' and 'scent mark' after the stressor, but these

did not reach significance (see Table 4.17). There was no effect of time of observation on behaviour (see Figure 4.9 and Table 4.18).

Figure 4.8 Mean sample points spent performing each behaviour before and after the stressor (untrained animals, collapsed across 1200, 1400 and 1600h) (bars represent standard errors)



**p<0.01

Table 4.17 Results of within-subjects ANOVAs of effects of stressor on all behaviours (untrained animals, collapsed across 1200, 1400 and 1600h)

Behaviour	F	p
Watch observer	4.57	0.09
Inactive, alert	0.99	0.37
Inactive (both categories)	33.06	<0.01**
Locomote	3.57	0.12
Forage	2.07	0.21
Self-scratch	25.97	<0.01**
Scent mark	2.30	0.19
Vocalise	0.07	0.81
In nest box	3.75	0.11

All d.f. = 1,5

Figure 4.9 Mean sample points spent performing each behaviour at three successive time periods (untrained animals, pre- and post-stressor data combined) (bars represent standard errors)

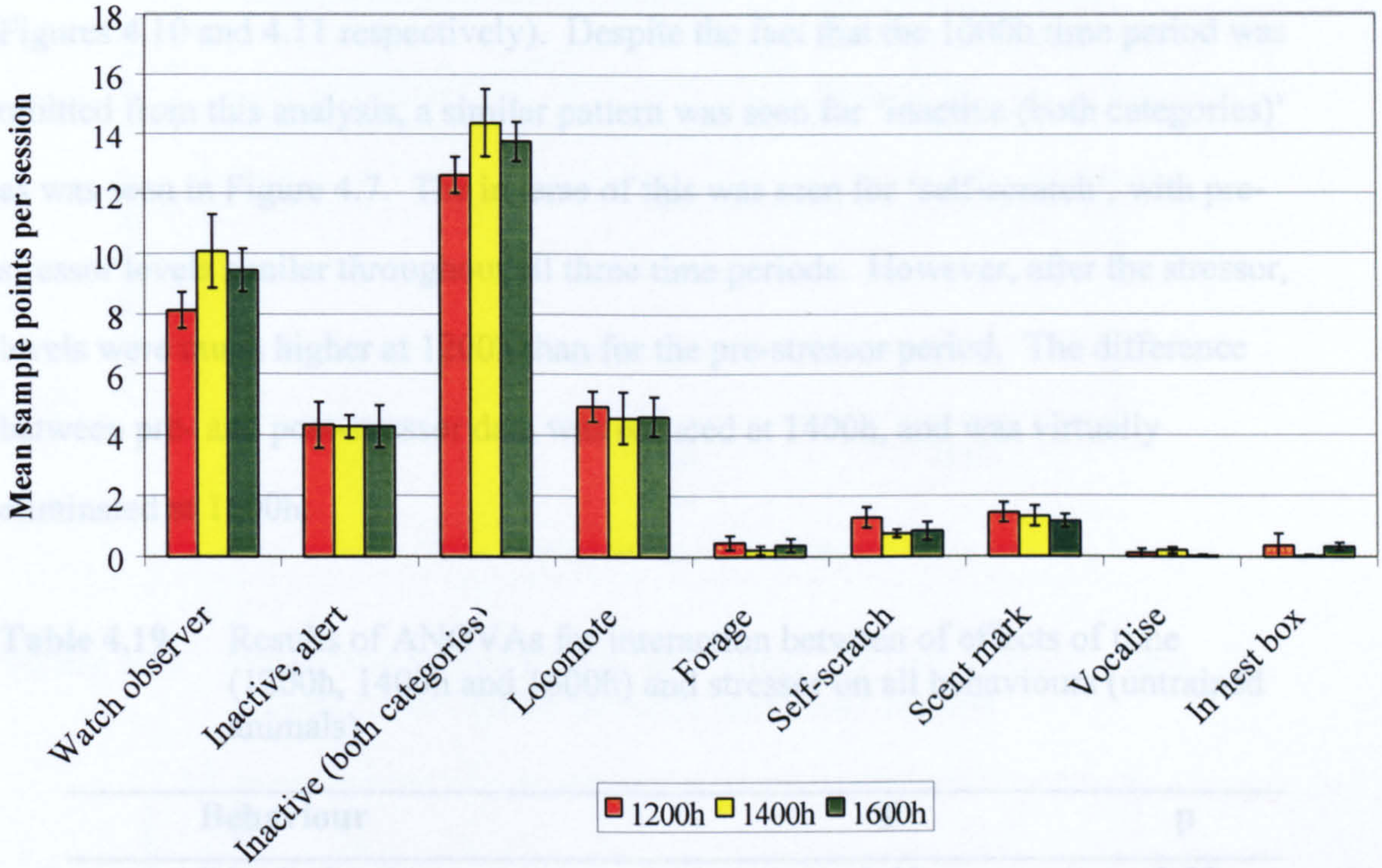


Table 4.18 Results of within-subjects ANOVAs of effects of time (1200h, 1400h and 1600h) on all behaviours (untrained animals, pre- and post-stressor combined)

Behaviour	F	p
Watch observer	3.91	0.06
Inactive, alert	0.00	1.00
Inactive (both categories)	3.16	0.09
Locomote	0.62	0.56
Forage	0.39	0.70
Self-scratch	1.16	0.35
Scent mark	0.57	0.58
Vocalise	0.71	0.51
In nest box	0.58	0.58

All d.f. = 2,10

The only behaviours that showed a significant interaction between 'time' and 'stress' were 'inactive (both categories)' and 'self-scratch' (see Table 4.19 and Figures 4.10 and 4.11 respectively). Despite the fact that the 1000h time period was omitted from this analysis, a similar pattern was seen for 'inactive (both categories)' as was seen in Figure 4.7. The inverse of this was seen for 'self-scratch', with pre-stressor levels similar throughout all three time periods. However, after the stressor, levels were much higher at 1200h than for the pre-stressor period. The difference between pre- and post-stressor data was reduced at 1400h, and was virtually eliminated at 1600h.

Table 4.19 Results of ANOVAs for interaction between of effects of time (1200h, 1400h and 1600h) and stressor on all behaviours (untrained animals)

Behaviour	F	p
Watch observer	1.43	0.28
Inactive, alert	1.48	0.27
Inactive (both categories)	4.72	<0.05*
Locomote	0.67	0.53
Forage	0.16	0.85
Self-scratch	9.83	<0.01**
Scent mark	0.78	0.49
Vocalise	1.82	0.21
In nest box	0.79	0.48

All d.f. = 2,10 * p<0.05; ** p<0.01

Figure 4.10 Interaction between 'time' and 'stress' for 'inactive (both categories)' – time periods 1200 – 1600h included (untrained animals)

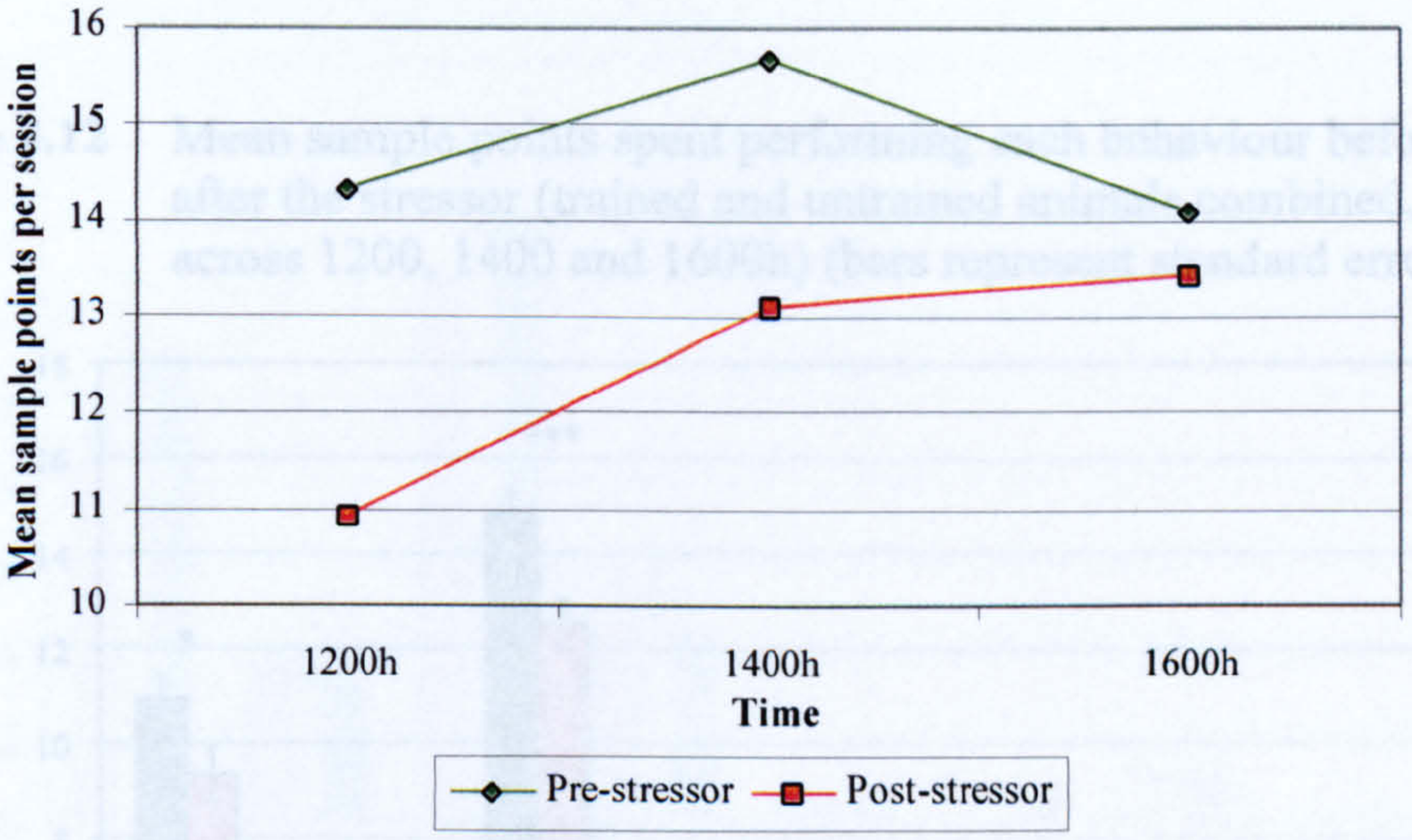
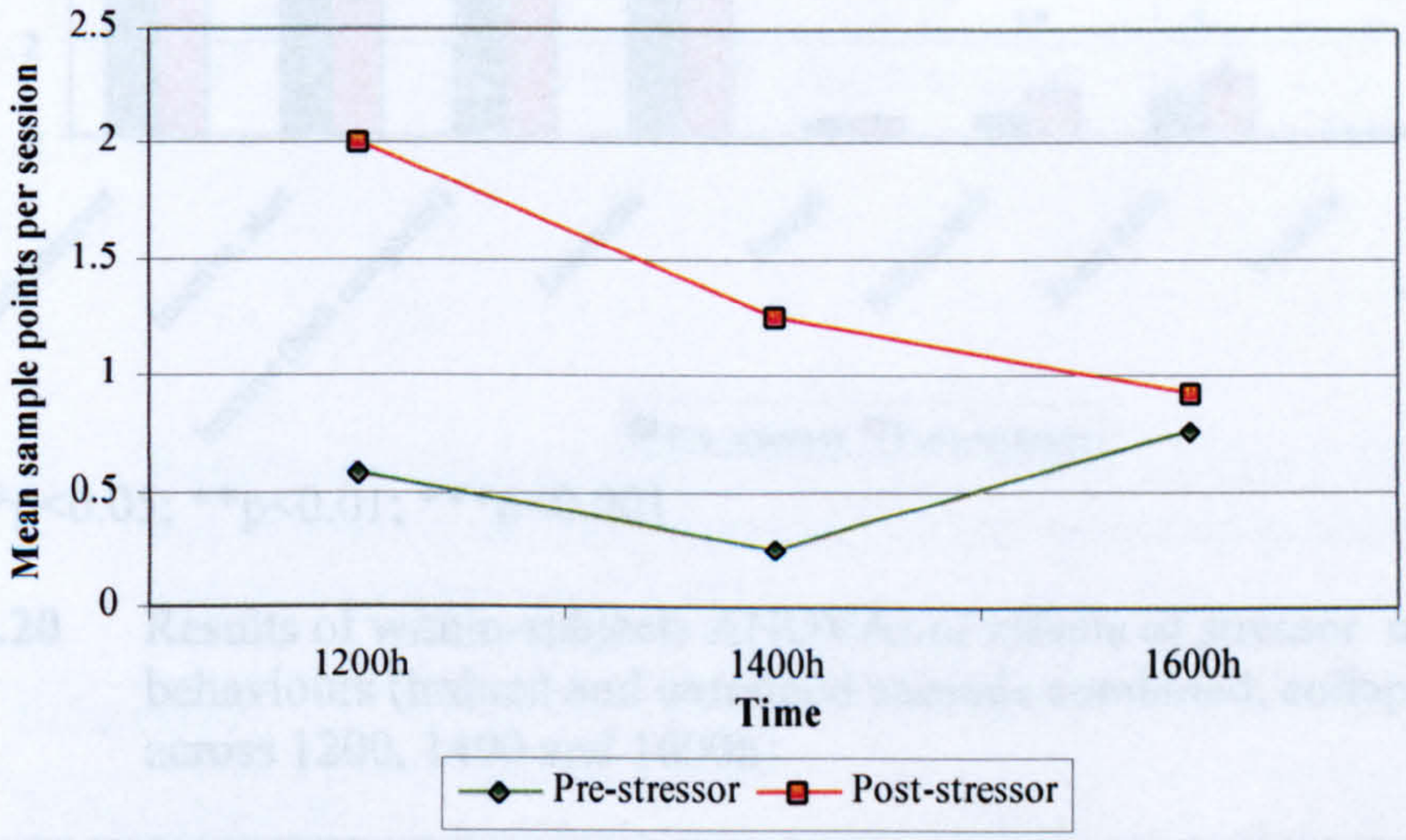


Figure 4.11 Interaction between 'time' and 'stress' for 'self-scratch' – time periods 1200 – 1600h included (untrained animals)



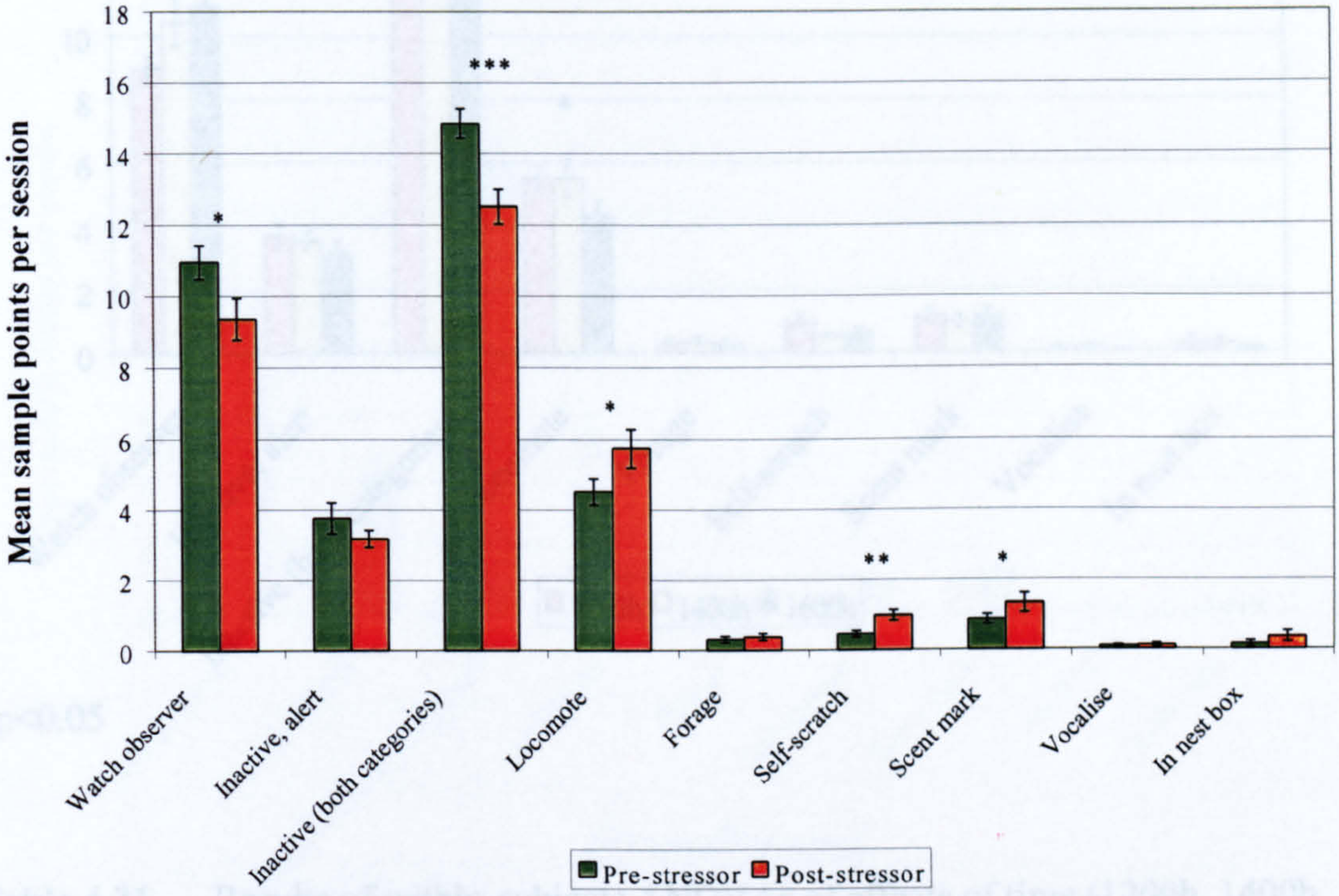
Trained and untrained animals combined

Effects of stressor

There was significantly less 'watch observer' and 'inactivity (both categories)' after the stressor compared with before it. There was also significantly more 'locomote', 'self-scratch' and 'scent mark' after the stressor compared with before it (see Figure 4.12 and Table 4.20). There was an effect of time of observation on 'watch observer', 'inactivity (both categories)' and 'locomote' (see Figure 4.13 and Table 4.21). Results of analyses of interactions for trained and

untrained animals combined are presented after the results of the main effects of each variable.

Figure 4.12 Mean sample points spent performing each behaviour before and after the stressor (trained and untrained animals combined, collapsed across 1200, 1400 and 1600h) (bars represent standard errors)



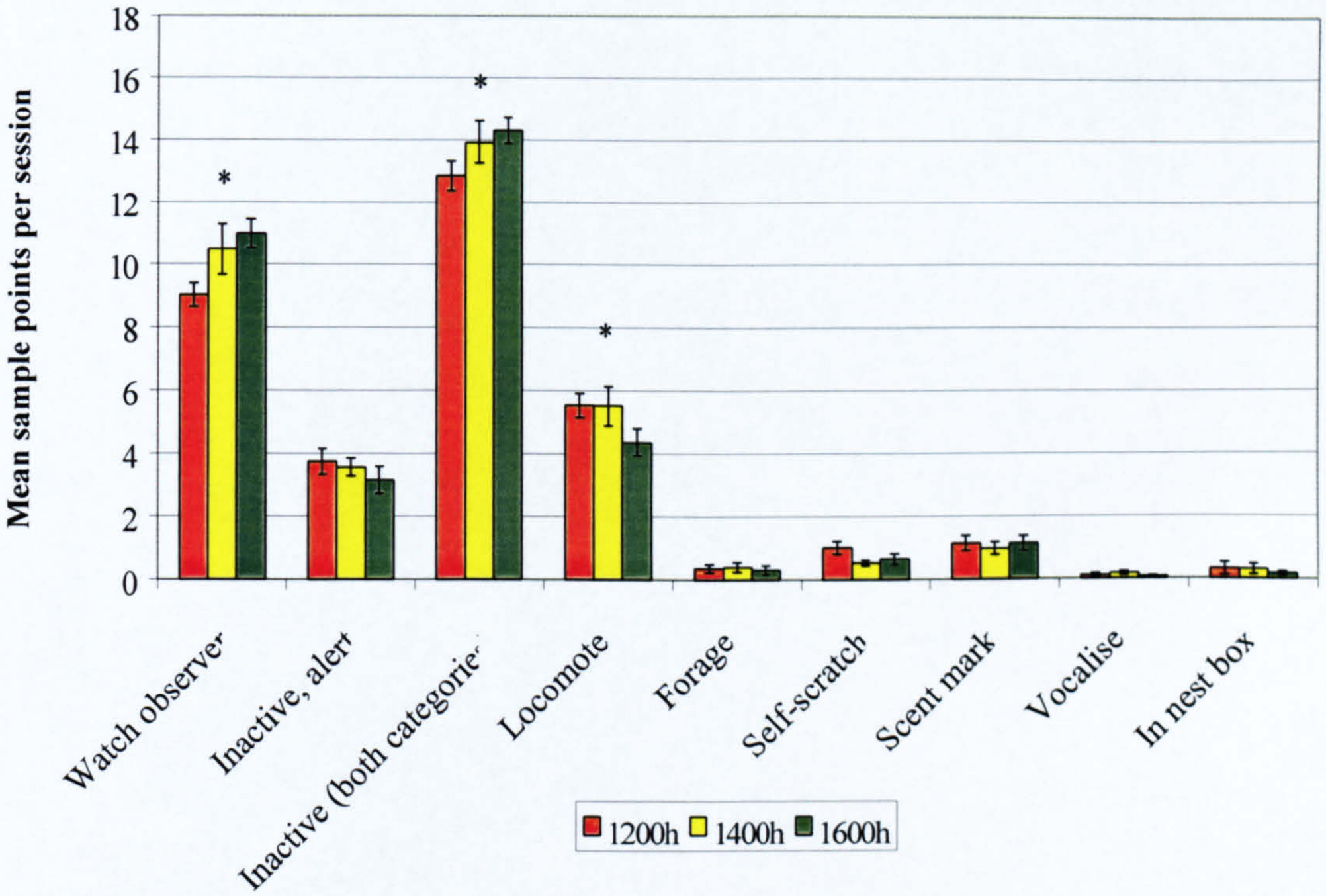
*p<0.05; **p<0.01; ***p<0.001

Table 4.20 Results of within-subjects ANOVAs of effects of stressor on all behaviours (trained and untrained animals combined, collapsed across 1200, 1400 and 1600h)

Behaviour	F	p
Watch observer	9.48	<0.05*
Inactive, alert	1.90	0.20
Inactive (both categories)	73.82	<0.001***
Locomote	7.08	<0.05*
Forage	0.20	0.66
Self-scratch	14.47	<0.01**
Scent mark	6.24	<0.05*
Vocalise	0.14	0.72
In nest box	1.74	0.22

All d.f. = 1,22 *** p<0.001; ** p<0.01; * p<0.05

Figure 4.13 Mean sample points spent performing each behaviour at three successive time periods (trained and untrained animals, pre- and post- stressor data combined) (bars represent standard errors)



*p<0.05

Table 4.21 Results of within-subjects ANOVAs of effects of time (1200h, 1400h and 1600h) on all behaviours (trained and untrained animals, pre- and post-stressor combined)

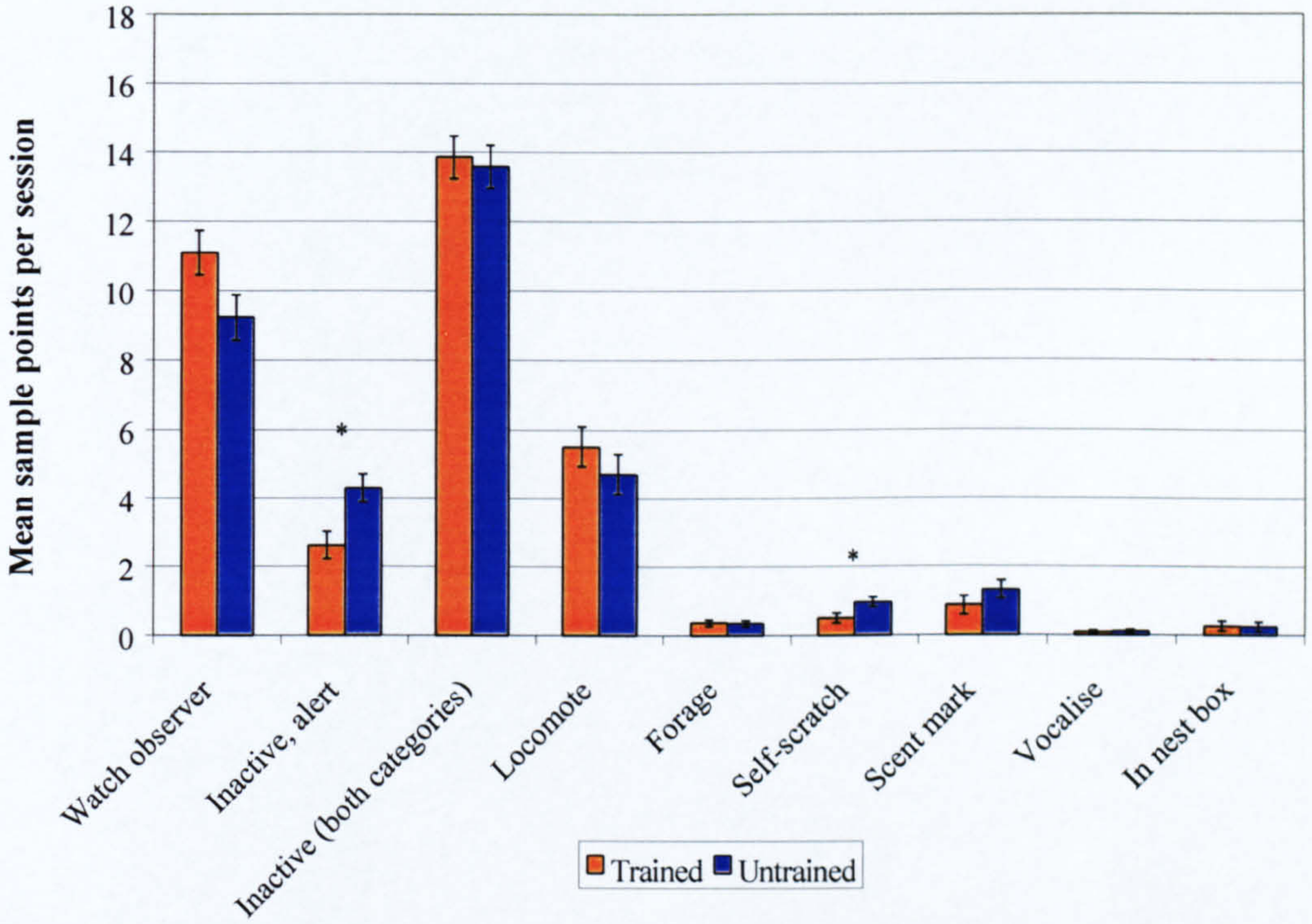
Behaviour	F	p
Watch observer	5.32	<0.05*
Inactive, alert	0.96	0.40
Inactive (both categories)	3.98	<0.05*
Locomote	4.12	<0.05*
Forage	0.08	0.93
Self-scratch	2.79	0.09
Scent mark	0.44	0.65
Vocalise	0.89	0.43
In nest box	0.28	0.76

All d.f. = 2,22 * p<0.05

Effect of Training

Frequencies of 'inactive, alert' and 'self-scratch' were significantly lower in trained than untrained animals (see Figure 4.14 and Table 4.22).

Figure 4.14 Mean sample points spent performing each behaviour for trained and untrained animals (collapsed across 1200, 1400 and 1600h) (bars represent standard errors)



*p<0.05

Table 4.22 Results of between-subjects ANOVAs of effects of 'training' on all behaviours (pre- and post-stressor combined, collapsed across 1200h, 1400h and 1600h)

Behaviour	F	p
Watch observer	4.23	0.07
Inactive, alert	8.33	<0.05*
Inactive (both categories)	0.14	0.72
Locomote	0.98	0.35
Forage	0.01	0.91
Self-scratch	5.17	<0.05*
Scent mark	1.46	0.25
Vocalise	0.20	0.66
In nest box	0.01	0.94

All d.f. = 1,10 * p<0.05

Interactions between the variables

1. 'Stress' and 'Time'

Both 'inactive, alert' and 'inactive (both categories)' showed significant interactions between 'stress' and 'time' (see Table 4.23 and Figures 4.15 and 4.16 respectively). Levels of 'inactivity (both categories)' remained relatively constant over time in the pre-stressor condition, whereas after the stressor they were decreased at 1200h, increasing over time until 1600h, by which time they were at a similar level to that of the pre-stressor condition. There was more inactivity while not watching the observer pre- stressor for the times 1200h and 1400h than there was post-stressor. However, at 1600h this pattern was reversed, with higher levels of the behaviour post-stressor. An interaction between 'stress' and 'time' was also found for 'self-scratch'. Pre-stressor rates remained similar throughout all the time periods. After the stressor, rates were much higher at 1200h, resulting in much higher rates than in the corresponding pre-stressor period. This difference decreased over time, and was virtually eliminated by 1600h. This was similar to the interaction for these variables seen for this behaviour for untrained animals (see Figure 4.11, page 121), but not for trained animals (see Table 4.11, page 111).

Table 4.23 Results of ANOVAs for interaction between effects of 'time' (1200h, 1400h and 1600h) and 'stress' on all behaviours (trained and untrained animals combined)

Behaviour	**F**	**p**
Watch observer	0.54	0.59
Inactive, alert	3.62	<0.05*
Inactive (both categories)	7.37	<0.01**
Locomote	1.57	0.23
Forage	0.13	0.88
Self-scratch	5.03	<0.05*
Scent mark	0.16	0.86
Vocalise	1.46	0.26
In nest box	0.30	0.75

All d.f. = 2,20

Figure 4.15 Interaction between 'stress' and 'time' for 'inactive, alert' (trained and untrained animals combined)

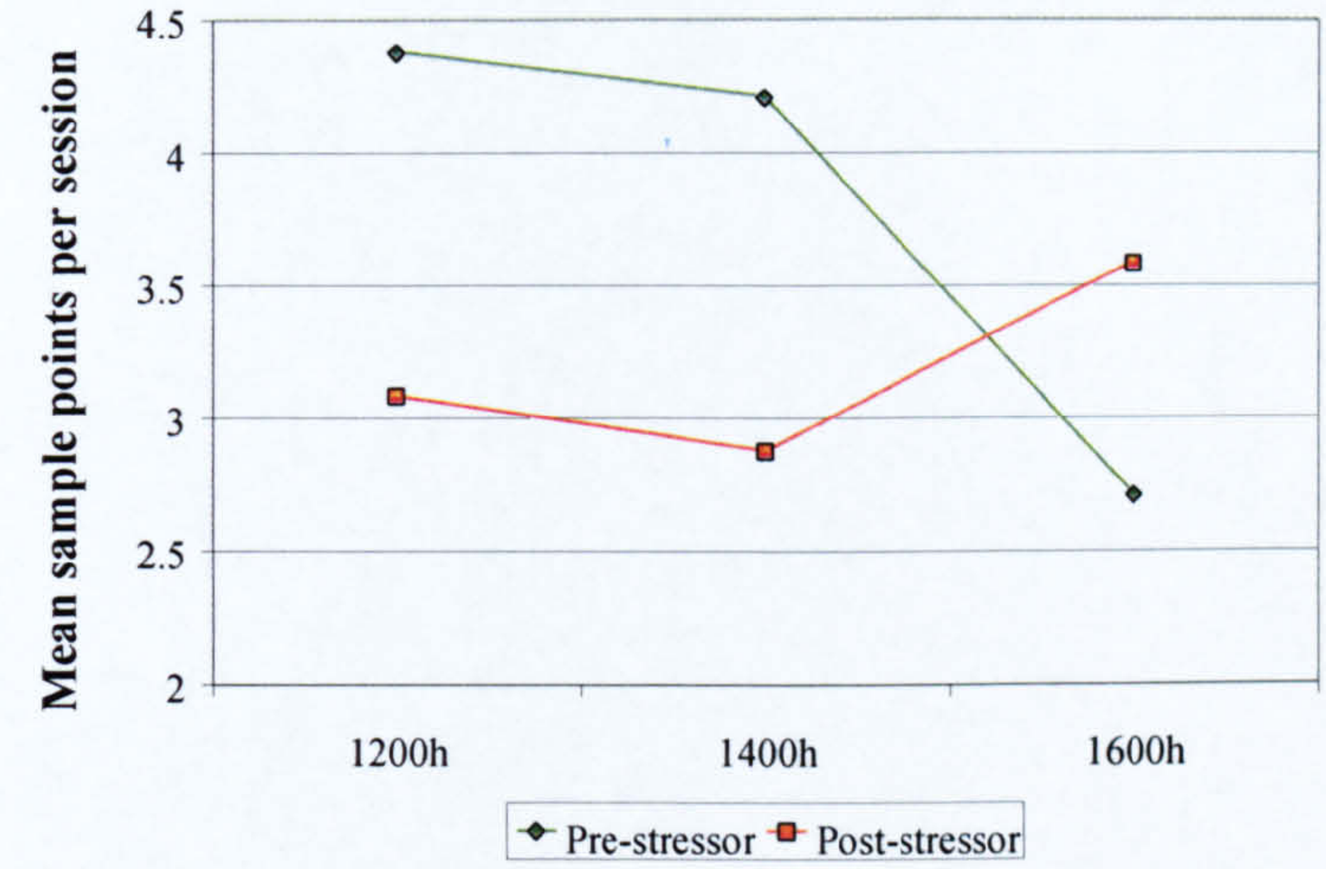


Figure 4.16 Interaction between 'stress' and 'time' for 'inactivity (both categories)' (trained and untrained animals combined)

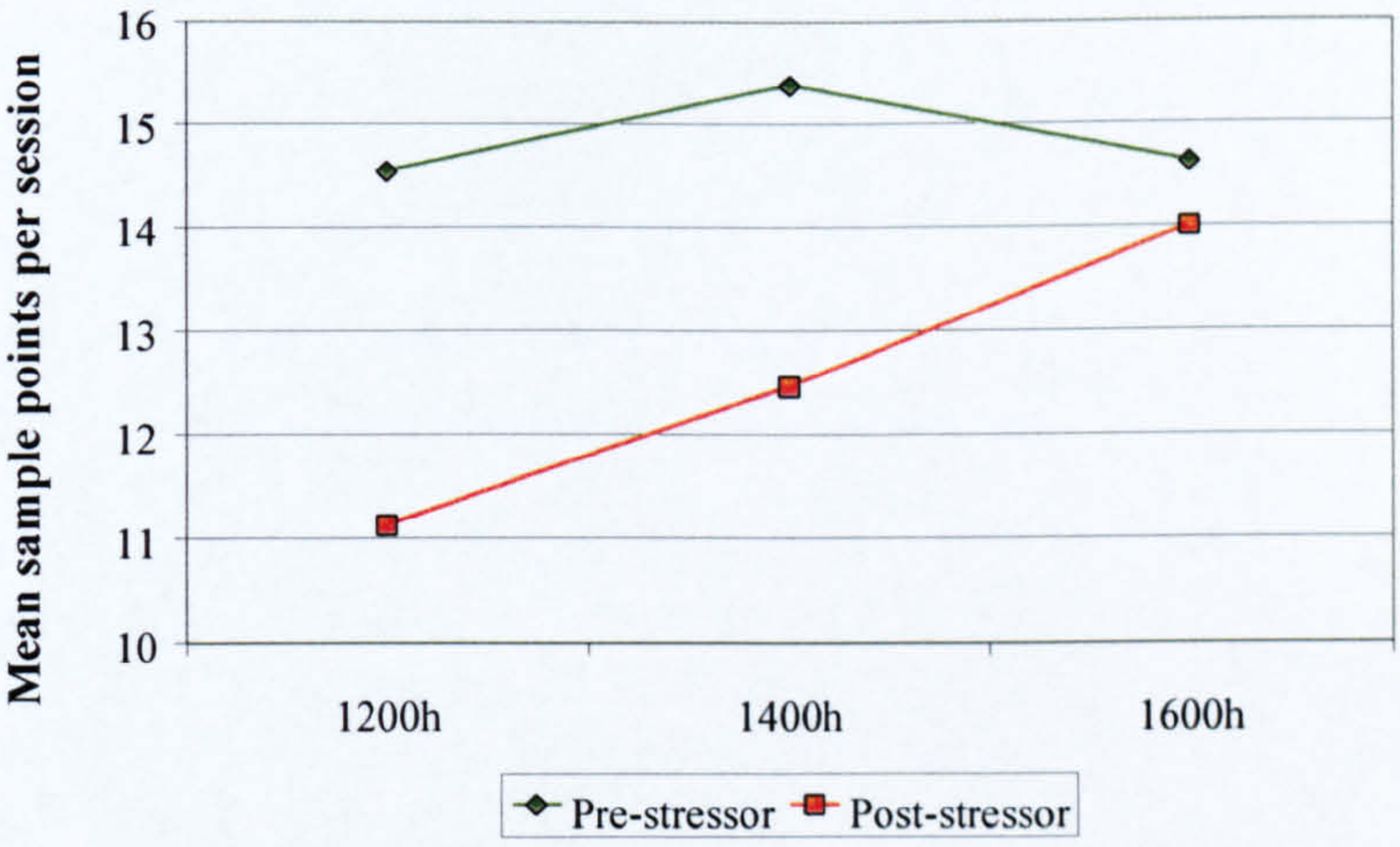
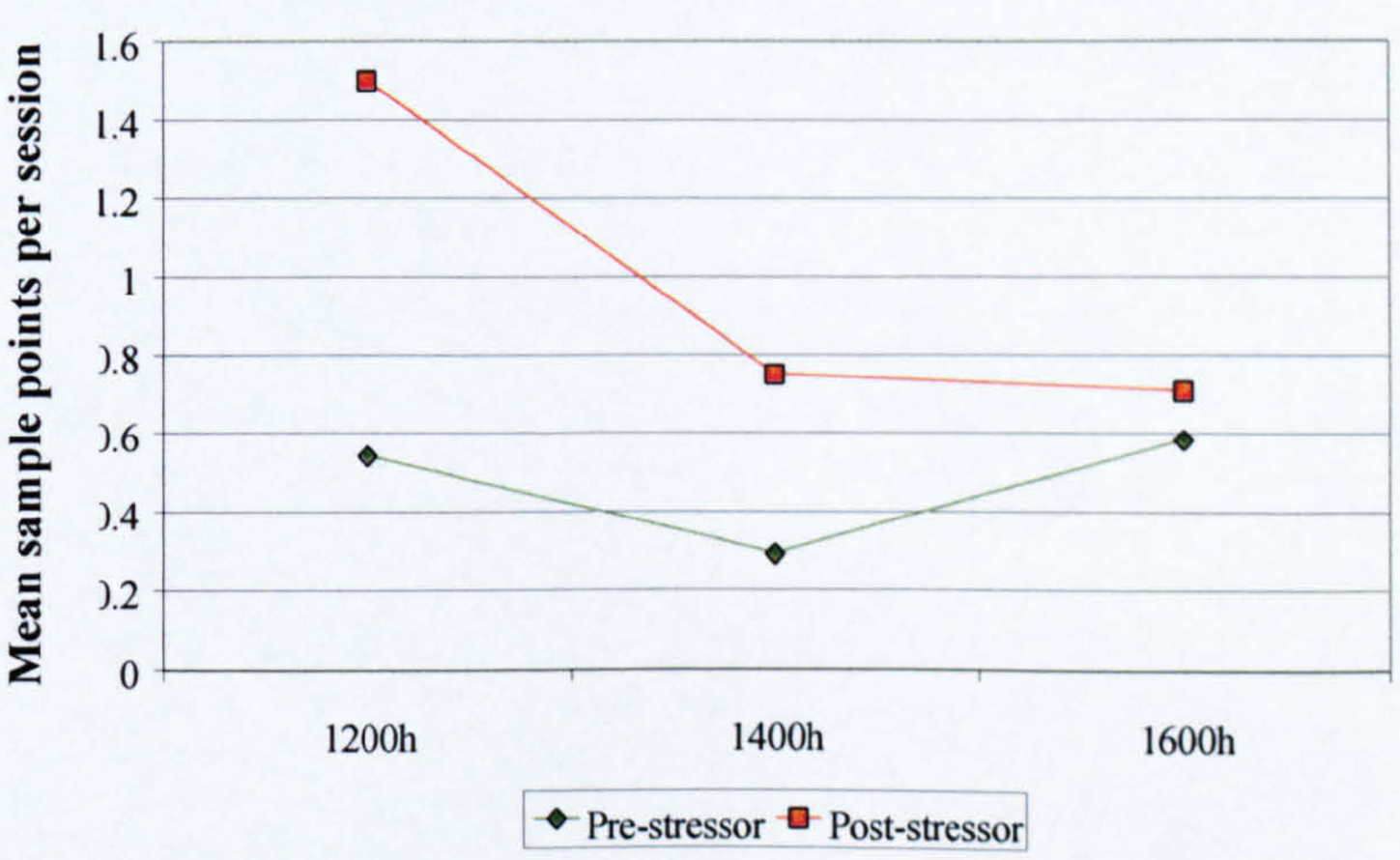


Figure 4.17 Interaction between 'stress' and 'time' for 'self-scratch' (trained and untrained animals combined)



2. 'Stress' and 'Training'

There were significant interactions between 'training' and 'stress' for 'forage' and 'self-scratch' (see Table 4.24 and Figures 4.18 and 4.19 respectively). For trained animals, there was more 'forage' after the stressor than before it. However, for untrained individuals, the opposite was seen; there was less 'forage' post-stressor. Although there was a very slight increase in the amount of 'self-scratch' seen in trained animals after the stressor, there was a large increase in the amount shown by untrained animals. The pre-stressor levels of 'self-scratch' were very similar for both groups, whereas after the stressor untrained animals scratched more than trained individuals.

Table 4.24 Results of ANOVAs for interaction between effects of 'stress' and 'training' on all behaviours (1200, 1400 and 1600h data combined)

Behaviour	F	p
Watch observer	0.21	0.66
Inactive, alert	0.04	0.85
Inactive (both categories)	<0.01	0.96
Locomote	0.72	0.42
Forage	5.95	<0.05*
Self-scratch	6.61	<0.05*
Scent mark	0.05	0.83
Vocalise	0.55	0.48
In nest box	0.07	0.80

All d.f. = 1,10

Figure 4.18 Interaction between 'training' and 'stress' for 'forage' – (collapsed across 1200, 1400 and 1600h)

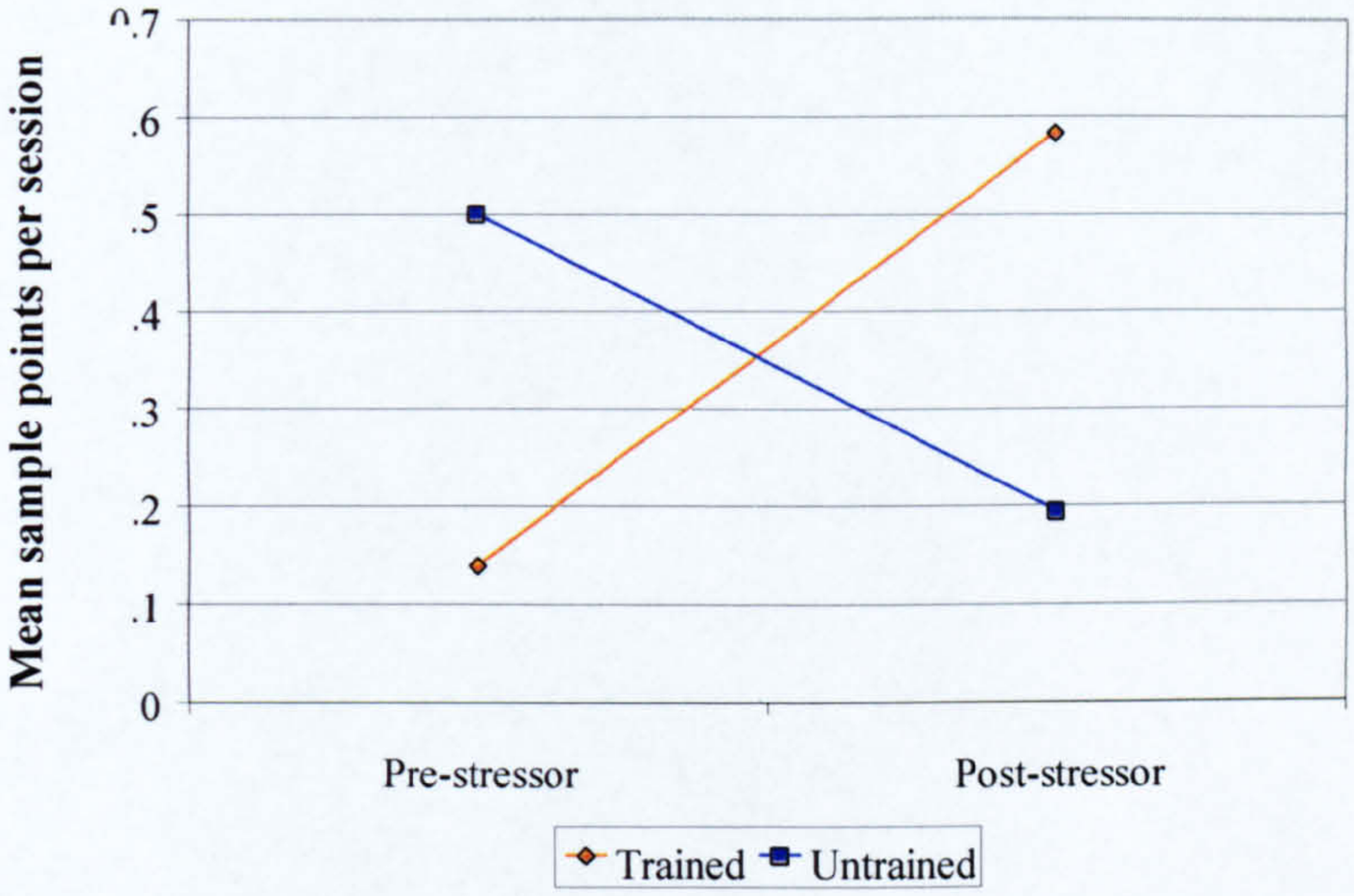
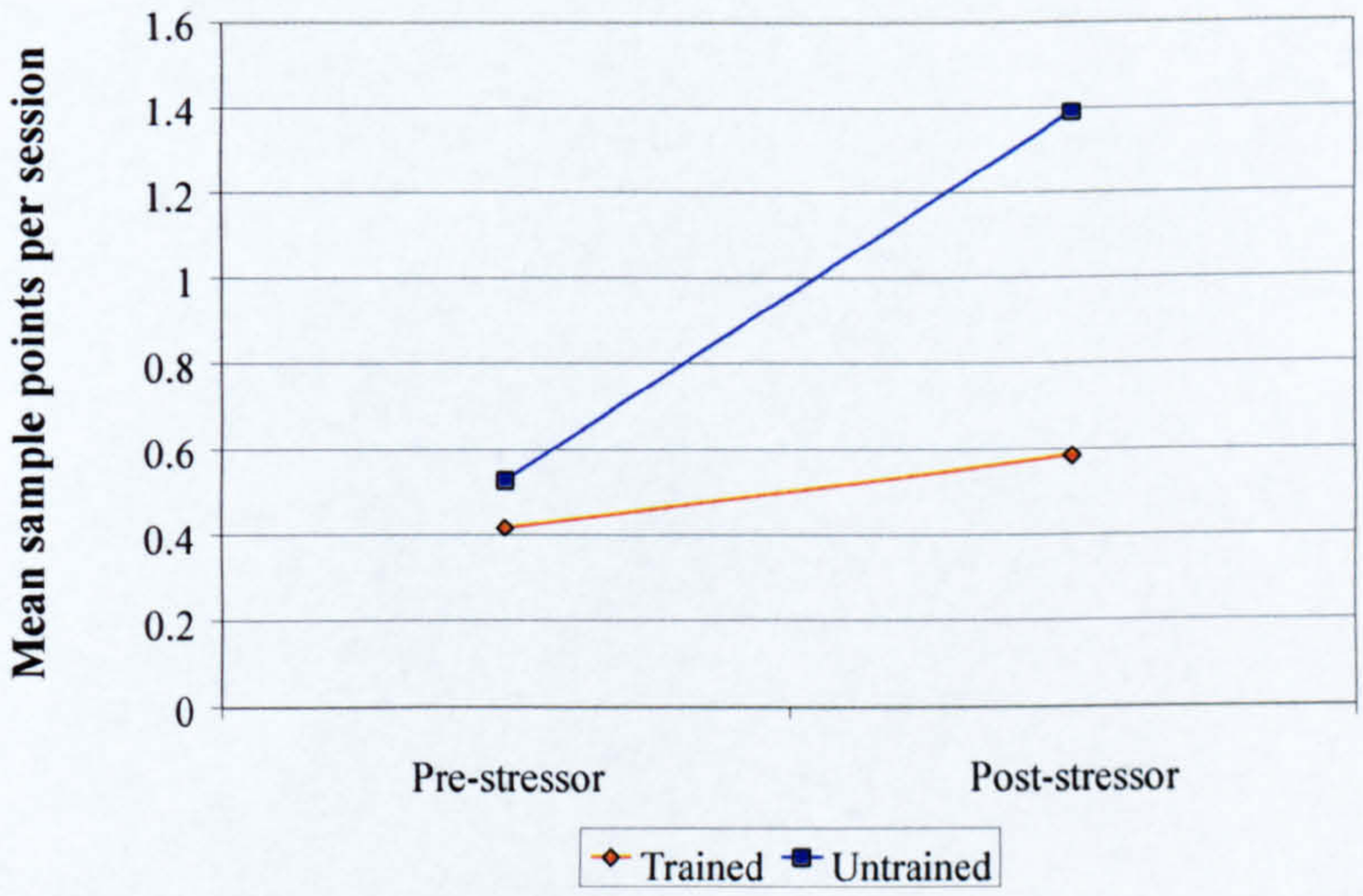


Figure 4.19 Interaction between 'training' and 'stress' for 'self-scratch' – (collapsed across 1200, 1400 and 1600h)



3. 'Time' and 'Training'

There were no significant interactions between 'training' and 'time' for any behaviour (see Table 4.25).

Table 4.25 Results of ANOVAs for interaction between effects of 'training' and 'time' (1200h, 1400h and 1600h) on all behaviours (pre- and post-stressor data combined)

Behaviour	F	p
Watch observer	1.63	0.22
Inactive, alert	0.85	0.44
Inactive (both categories)	1.25	0.31
Locomote	3.44	0.06
Forage	1.10	0.35
Self-scratch	0.07	0.93
Scent mark	1.56	0.23
Vocalise	0.33	0.73
In nest box	2.14	0.14

All d.f. = 2,20

4. 'Stress', 'Training' and 'Time'

There were no three-way interactions between 'training', 'stress' and 'time' (see Table 4.26).

Table 4.26 Results of ANOVAs for three-way interaction between effects of 'training', 'stress' and 'time' (1200h, 1400h and 1600h) on all behaviours

Behaviour	F	p
Watch observer	<0.01	1.00
Inactive, alert	0.62	0.55
Inactive (both categories)	0.68	0.52
Locomote	1.66	0.22
Forage	<0.01	1.00
Self-scratch	2.05	0.16
Scent mark	1.09	0.35
Vocalise	1.46	0.26
In nest box	1.06	0.37

All d.f. = 2,20

4.3.2 Results of cortisol analyses

1. Circadian rhythms

There were no significant main effects of time or sex on urinary cortisol concentration ($F_{7,49}$=0.57; p=0.78 and $F_{1,7}$=0.68; p=0.44) (see Figure 4.20). There was also no significant interaction between time and sex ($F_{7,49}$=0.68; p=0.44). When data for each sex were analysed separately, there was still no significant effect of time for males or females ($F_{7,28}$=0.72; p=0.66; $F_{7,21}$=0.62; p=0.71 respectively) (see Figure 4.21).

Figure 4.20 Mean pre-stressor concentration of urinary cortisol per hour (bars represent standard errors)

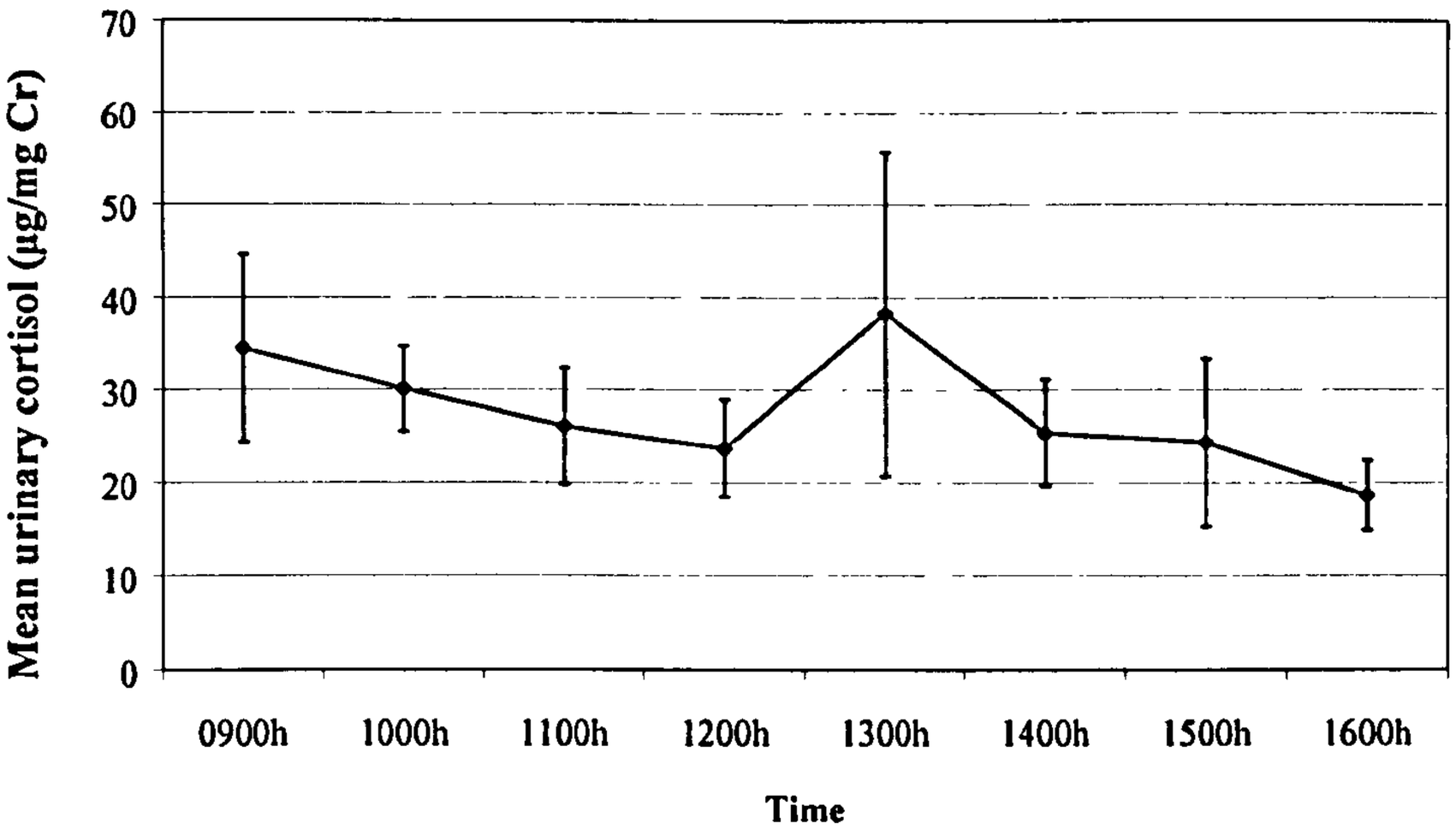
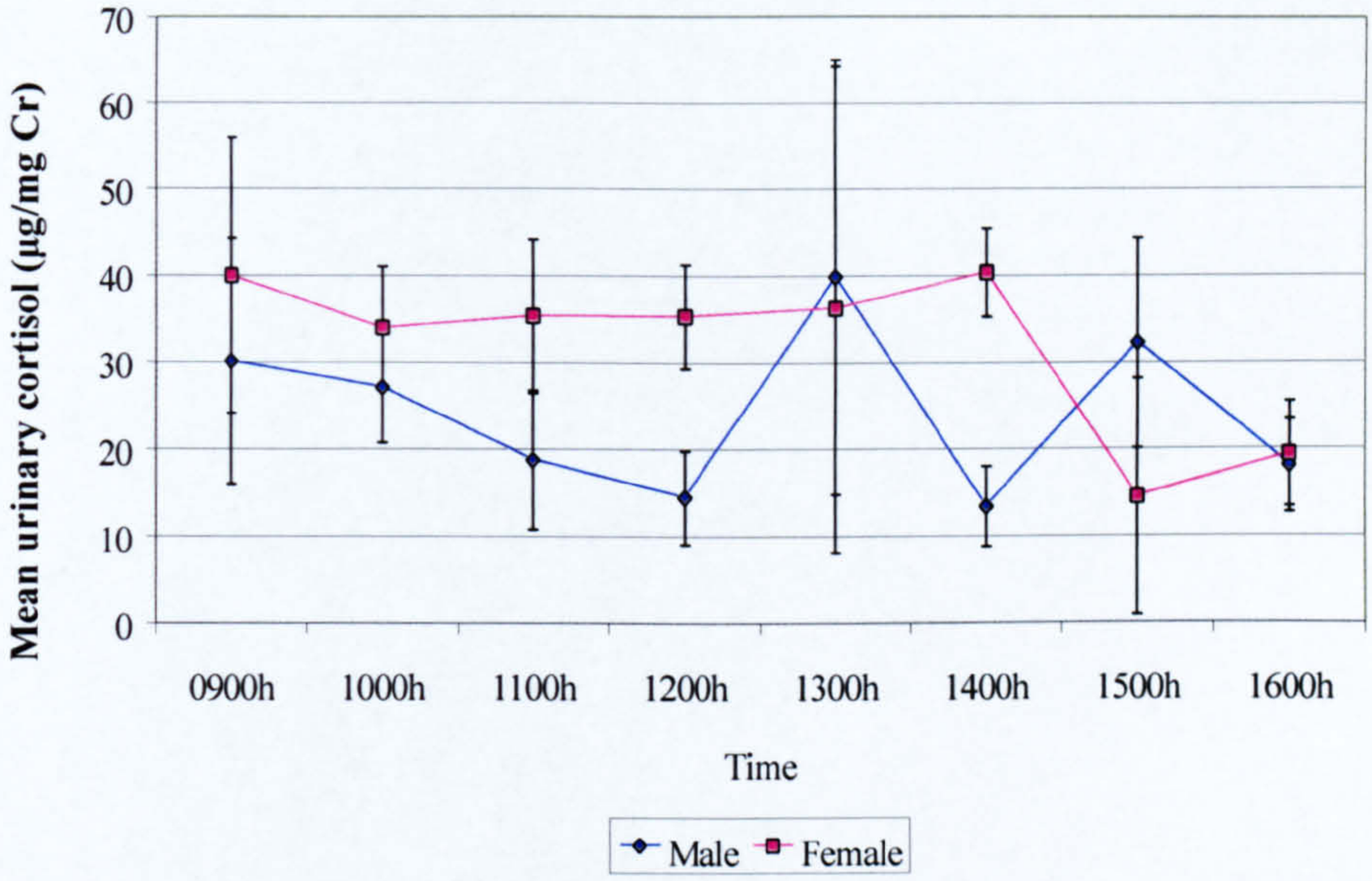


Figure 4.21 Mean pre-stressor concentration of urinary cortisol per hour for male and female animals (bars represent standard errors)



When data were collapsed into 2 hour time blocks, there were still no significant main effects of time or sex on urinary cortisol concentration ($F_{3,30}$=2.50; p=0.08 and $F_{1,10}$=0.33; p=0.57 respectively). Neither was there a significant interaction between time and sex ($F_{3,30}$=0.92; p=0.36). However, there appeared to be a trend of decreasing urinary cortisol throughout the day (see Figure 4.22). When data for each sex were analysed separately, there was still no significant effect of time for males ($F_{3,15}$=0.88; p=0.47). However, a significant effect of time was found for females ($F_{3,15}$=5.60; p<0.01). In females, cortisol concentrations for the first three time blocks were reduced slightly over time, but there was a sharp reduction in cortisol for the last time period (see Figure 4.23). Cortisol concentrations at 0900-1000h were significantly higher than those at 1500-1600h for females (see Table 4.27).

Figure 4.22 Mean pre-stressor concentration of urinary cortisol per 2-hour time period (bars represent standard errors)

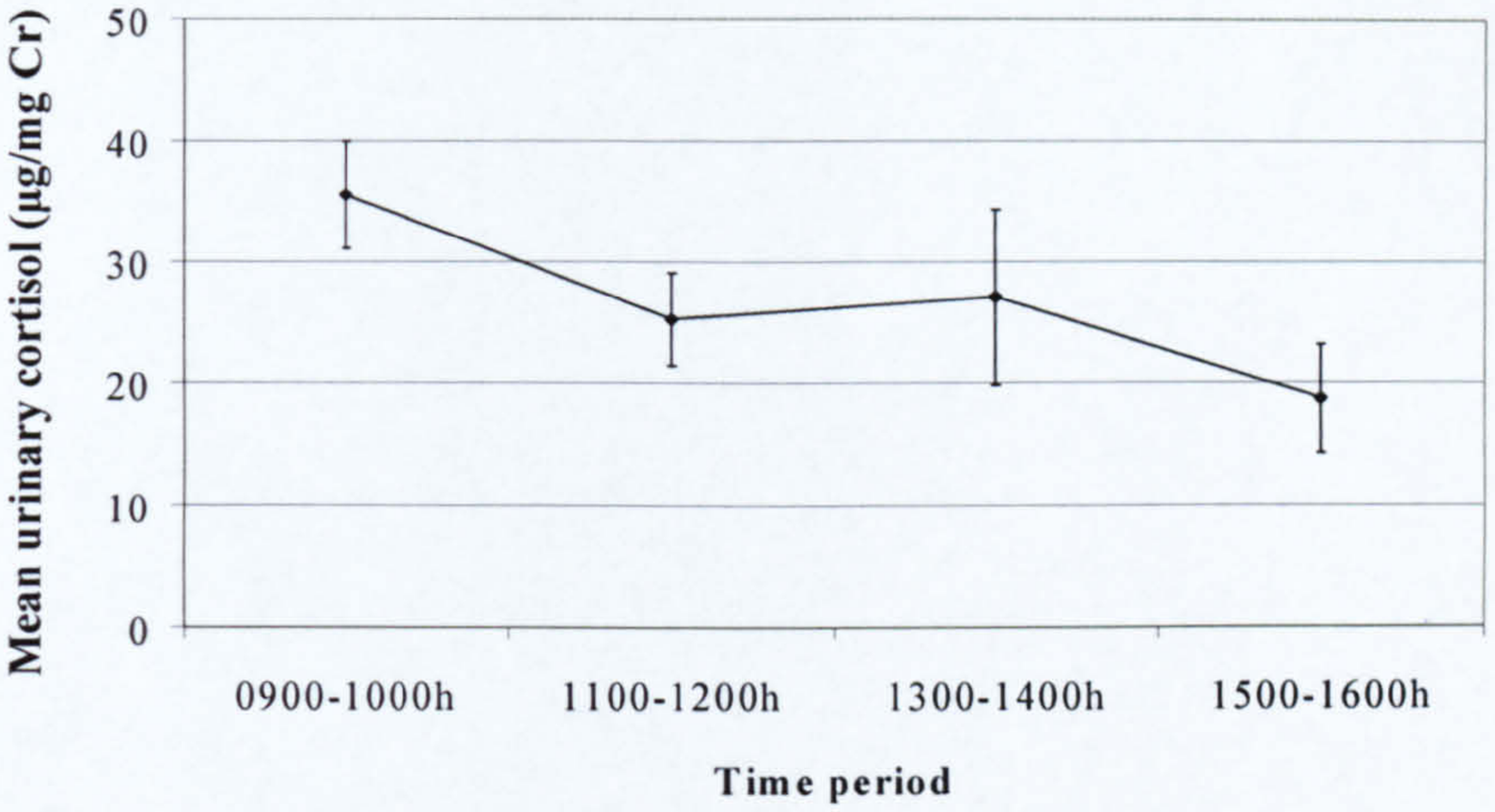


Figure 4.23 Mean pre-stressor concentration of urinary cortisol per 2-hour block for male and female animals (bars represent standard errors)

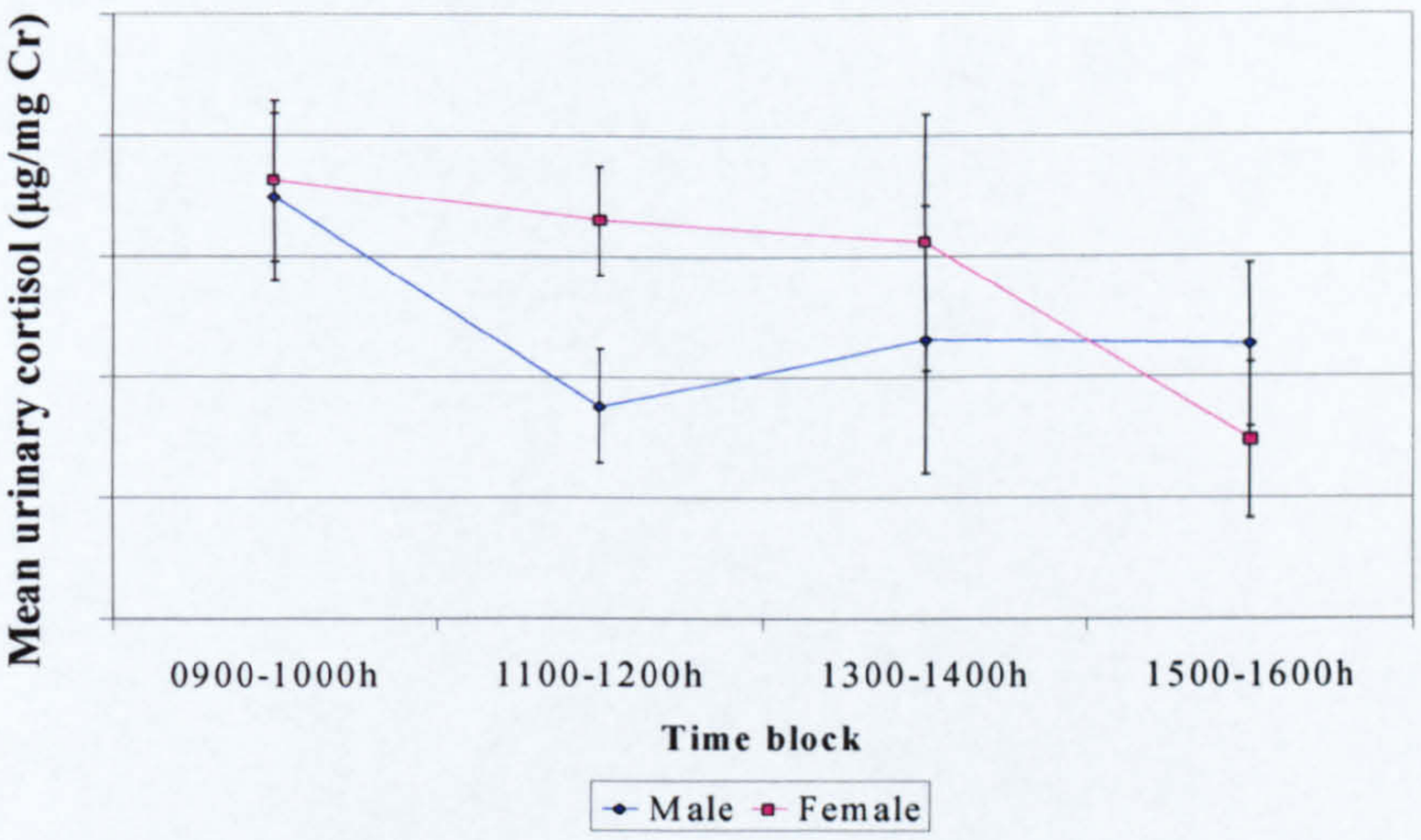


Table 4.27 Post-hoc *t*-test *t* and p values for mean pre-stressor concentration of urinary cortisol per 2-hour time blocks (females only)

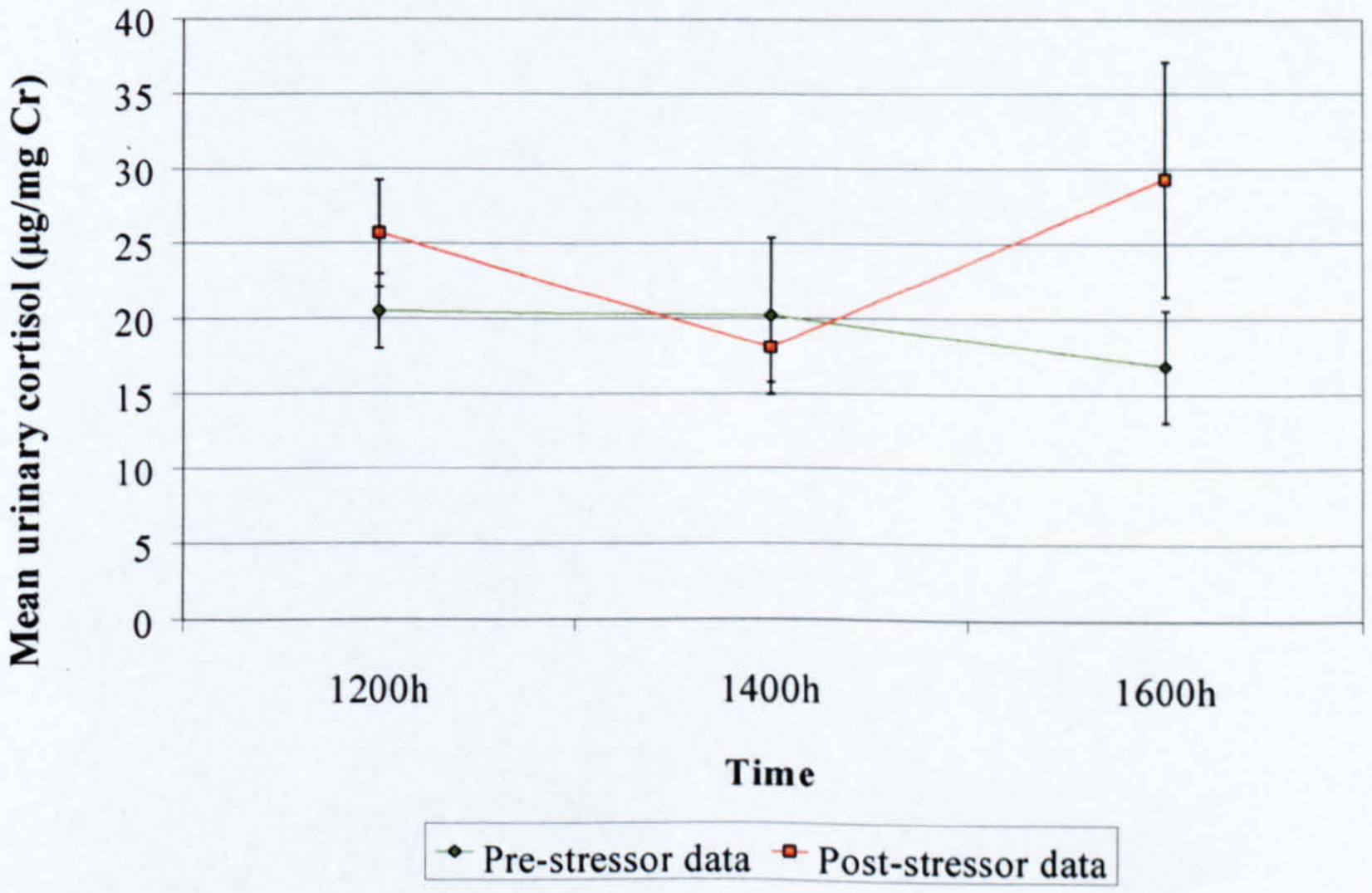
Time Blocks	***t***	**p** (uncorrected)	**p** (following Bonferroni correction)
0900-1000h vs. 1100-1200h	0.61	0.57	1.00
0900-1000h vs. 1300-1400h	0.53	0.62	1.00
0900-1000h vs. 1500-1600h	5.78	<0.01**	<0.05*
1100-1200h vs. 1300-1400h	0.09	0.93	1.00
1100-1200h vs. 1500-1600h	3.87	<0.05*	0.07
1300-1400h vs. 1500-1600h	2.35	0.08	0.47

All d.f. = 5 ** p<0.01; * p<0.05

2. Response to a stressor

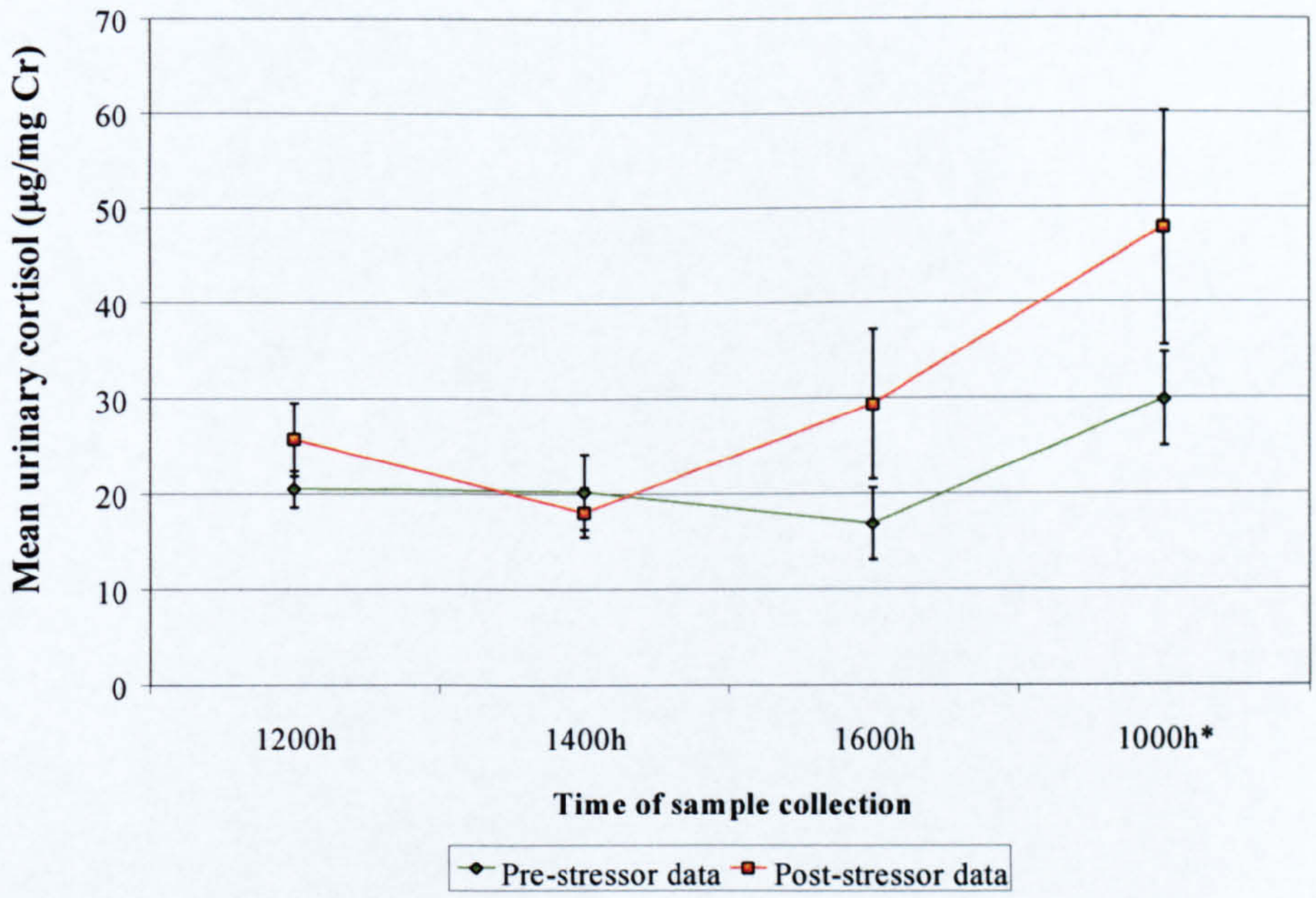
When pre-stressor data were compared only with data collected on stressor day (3 time periods only), there were no significant effects of time or stress on urinary cortisol ($F_{2,18}$=0.92; p=0.42 and $F_{1,9}$=4.45; p=0.06 respectively) (see Figure 4.24).

Figure 4.24 Mean concentrations of urinary cortisol at three pre-stressor time periods and at the same three time periods on the day of administration of a mild stressor (stressor given at 1000h) (bars represent standard errors)



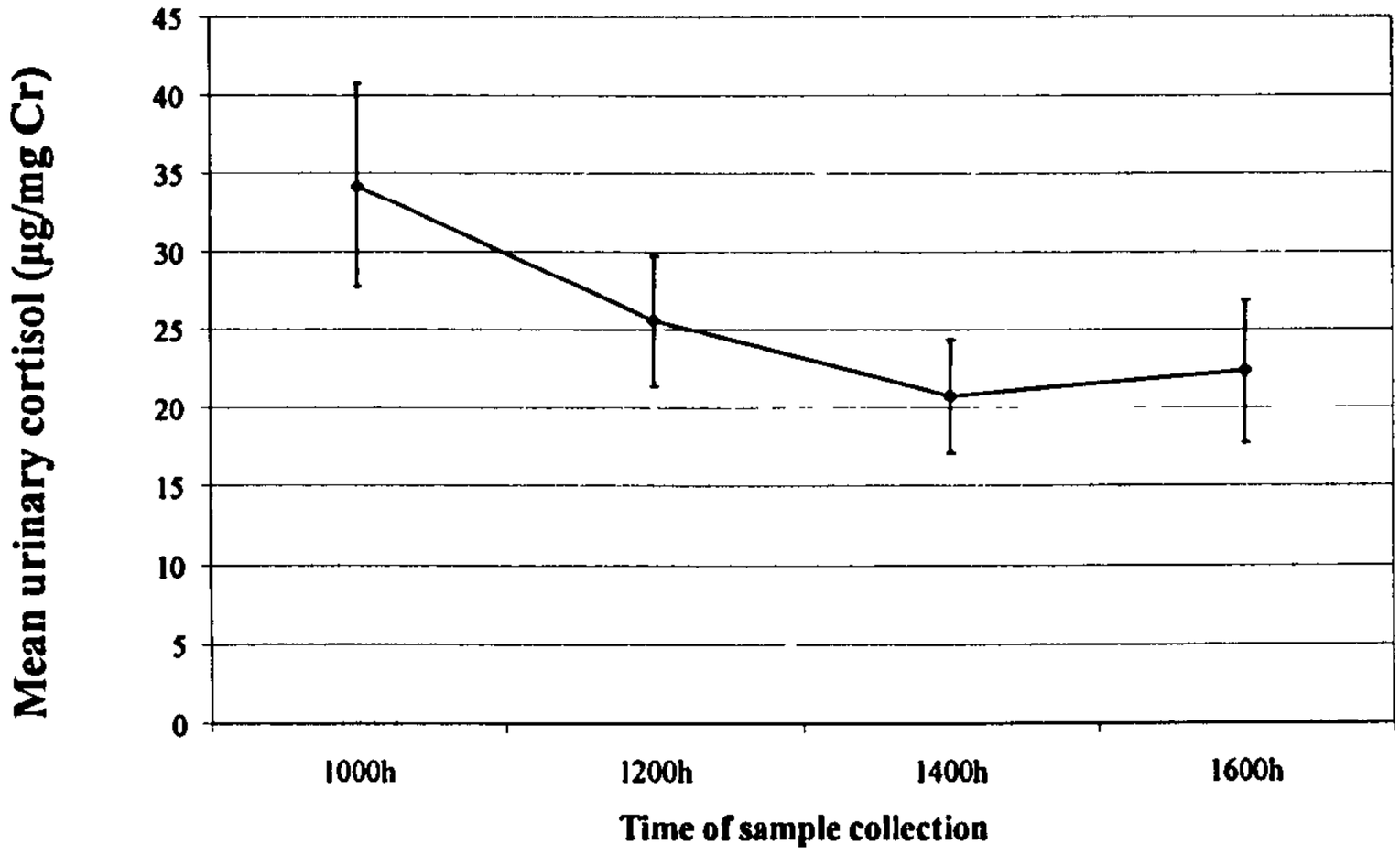
When pre-stressor 1000h cortisol values were matched with 1000h values from the day after stressor administration and included in the analysis, there was still no significant effect of stress on urinary cortisol ($F_{1,8}$=3.59; p=0.10). There was, a significant effect of time on cortisol concentration ($F_{3,24}$=3.03; p<0.05) (see Figure 4.25). However, post-hoc tests revealed that mean cortisol concentrations were not significantly different to each other at any of the individual time periods (see Figure 4.26 and Table 4.28).

Figure 4.25 Mean concentrations of urinary cortisol pre-stressor and following administration of a mild stressor (administered at 1000h) (bars represent standard errors)



*1000h sample was collected the day after the administration of the stressor for the post-stressor data

Figure 4.26 Mean concentrations of urinary cortisol at four different time periods (pre- and post-stressor combined) (bars represent standard errors)



*1000h sample was collected the day after the administration of the stressor for the post-stressor data

Table 4.28 Post-hoc *t*-test *t* and p values for concentration of urinary cortisol at four different time periods (male and female animals, pre- and post-stressor values combined)

Time periods	***t***	**p** (uncorrected)	**p** (following Bonferroni correction)
1000h vs. 1200h	2.33	<0.05*	0.24
1000h vs. 1400h	2.40	<0.05*	0.21
1000h vs. 1600h	2.05	0.07	0.39
1200h vs. 1400h	1.59	0.14	0.83
1200h vs. 1600h	0.74	0.48	1.00
1400h vs. 1600h	0.45	0.66	1.00

All d.f. = 11 * $p<0.05$

There was no significant effect of day when concentrations of urinary cortisol collected at 1000h before the stressor and 1, 2, 5 and 7 days after the stressor were compared ($F_{4,32}=1.93$; $p=0.13$) (see Figure 4.27). When data for males and females were analysed separately, there was still no effect of day on cortisol

concentration ($F_{4,12}$=1.64; p=0.23 and $F_{4,16}$=1.25; p=0.33 respectively) (see Figure 4.28).

Figure 4.27 Mean concentrations of urinary cortisol at 1000h pre-stressor and on four different days following administration of a mild stressor (bars represent standard errors)

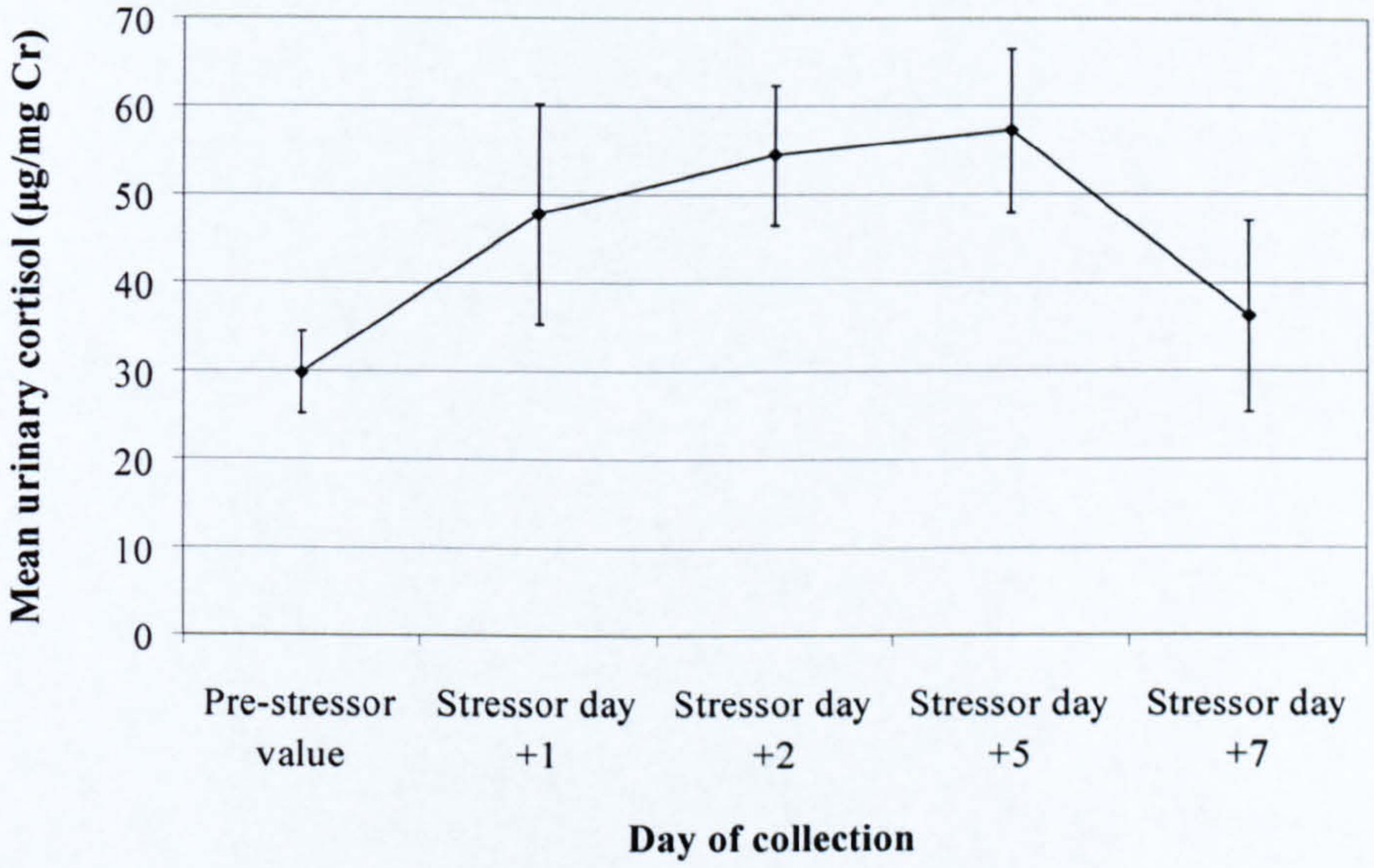
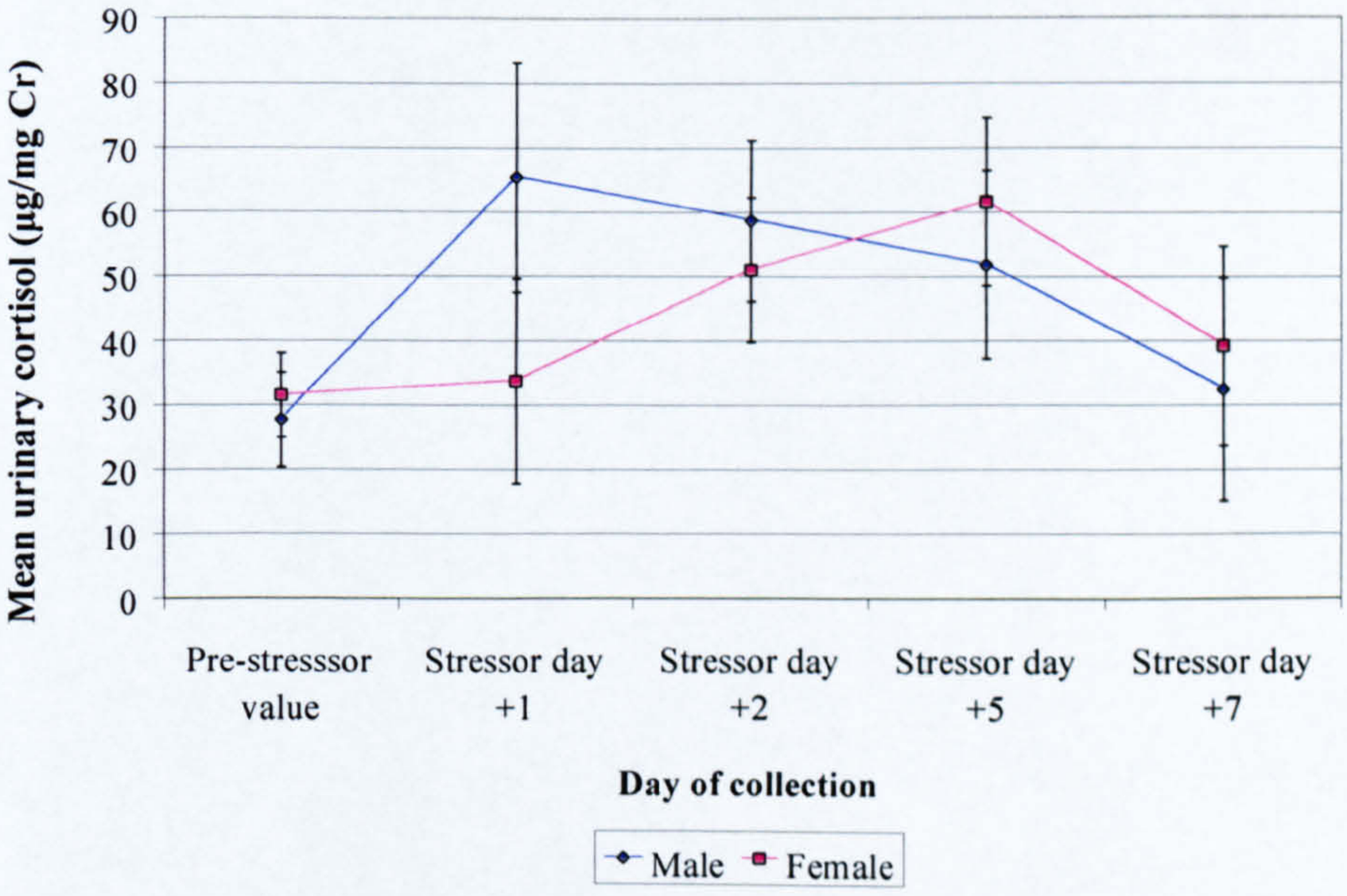


Figure 4.28 Mean concentrations of urinary cortisol at 1000h pre-stressor and on four different days following administration of a mild stressor, shown for male and female animals separately (bars represent standard errors)



4.4 DISCUSSION

The study showed that in untrained marmosets, frequencies of several behaviours were altered following administration of a stressor. Animals displaying similar changes in these behaviours in other situations may be stressed, with their welfare at risk as a result. The use of urinary cortisol as an indicator of stress in common marmosets was not supported by data from this study. Evidence of circadian variation on urinary cortisol excretion was only seen for females, which showed a decrease in cortisol concentration over the day when data were pooled into 2-hour blocks.

Effects of diurnal variation on behaviour

A 24 hour cycle affecting physiological and behavioural mechanisms is commonly found in living organisms (Pittendrigh, 1981). Common marmosets are strictly diurnal, and their daily activity is entirely confined to light time (Erkert, 1989). In the present study, no behaviours were significantly affected by time of day, when data were analysed per hour. When data were pooled into two-hour time blocks, 'inactive, alert' was affected by time of day. Rates of this behaviour were lower between 1100h and 1400h than at 1500-1600h. Most husbandry procedures were carried out in the morning, followed by feeding at around 12.45h, and after this it was usual for staff only to enter the marmoset rooms late in the afternoon for a final check of the animals. Therefore, the decrease in time spent inactive and alert but not watching the observer may be due to the fact that personnel being in the room, for lengthy periods, at this time was a fairly unusual occurrence for the animals. This may have resulted in increased arousal, and a reduction in inactive behaviour. However, the combined category of inactive behaviour was not significantly affected by time of day. This suggests that the animals may have spent more time vigilant and watching the observer in the afternoon at 1500-1600h, although this difference was not significant.

The fact that there was no diurnal variation in frequency of scent marking is in contrast to findings by Nogueira and co-workers (2001), who showed that scent marking in common marmosets varied significantly during the day, peaking between 1500h and 1700h, and being lowest at 1100h-1300h. However, these researchers observed scent marking over a longer time period (0500h-1700h) than was practicable in this study (where it was only possible to make observations between 0900h and 1600h). It is possible that had the animals in the present study been observed for a longer time period, significant results may have been obtained. Evidence for diurnal variation on the frequency of drinking (Saito *et al*, 1983) and grooming (Azevedo *et al*, 1996) has been reported in this species. It was unfeasible to attempt to test for comparable effects on the data available for this population, as the frequency of observations of these behaviours was so low. It is more surprising that I did not find a circadian effect on locomotor activity, as has been reported for common marmosets previously (Erkert, 1989; Menezes *et al*, 1993). However, captive common marmosets do show considerable inter-individual variation in activity patterns (Erkert, 1989). This variation in activity pattern has also been reported in common marmosets in the wild (Hubrecht, 1985); the activity pattern of wild marmosets also varies considerably from day to day (Maier *et al*, 1982). Individual variation, combined with the relatively small sample size and short time-scale of observations, may be responsible for the lack of significance in circadian rhythm patterns found in this study. It is also possible that the housing conditions, such as the small cage sizes, may have modified 'typical' behaviour patterns.

The fact that there may be diurnal variation in behavioural patterns in this species highlights the importance of collecting baseline data at a similar time period to experimental data. This would increase the validity of behavioural observations and applies in relation to any experimental manipulation, and not only to studies involving stressors such as this one.

Effects of a stressor on behaviour

Inactive (both categories)

Inactivity can be a major problem for many captive animals when kept in unstimulating environments (Erwin & Deni, 1979; McGrew, 1981). Studies on environmental enrichment for captive primates tend to consider an increase in activity to be beneficial, especially where individuals are initially inactive and apathetic (Laule & Desmond, 1998; Shepherdson, 1989). Conversely, animals displaying hyperactivity (*e.g.* pacing) may benefit from having activity levels reduced (Shepherdson, 1989).

There was a significant reduction in inactivity following administration of the stressor for both trained and untrained animals. Inactivity increased over the day for the trained animals, when data collected at 1200h, 1400h and 1600h, pre- and post-stressor data combined were analysed. However, this trend disappeared when data collected hourly throughout the whole day were used in the analysis. Inactivity also increased over the day for untrained animals, for data collected at 1000h, 1200h, 1400h and 1600h, pre-and post-stressor data combined. The significant interaction between 'stress' and 'time' seen for untrained animals may be responsible for this effect. Untrained animals showed a relatively constant rate of inactivity (both categories) pre-stressor, but rates of this behaviour were very low immediately (1000h) post-stressor, gradually increasing during the day. The effect of the stressor on this behaviour is therefore strongest immediately following its administration, and returns to baseline (pre-stressor) levels by 1600h. This effect on post-stressor behaviour appears to be so strong as to affect the combined results of the two sets of data (pre- and post-stressor), thus there appears to be a general increase in inactivity (both categories) over the day. This effect is not seen in the pre-stressor data (trained animals only) collected hourly throughout the day. A similar effect may be responsible for the positive result found for the trained

animals when pre- and post-stressor behaviour was combined. However, here there was no significant interaction between the variables of 'stress' and 'time'.

The most interesting effect here, however, was that administration of the stressor resulted in a decrease in inactivity (both categories) in both trained and untrained animals. This behaviour was the only one that was significantly affected by the stressor for the trained animals. It is possible, therefore, that a decrease in the amount of time spent inactive may be the most sensitive measure of stress for this species. Inactivity may indeed be affected by factors that would seem to impair welfare, such as illness, fear or extremes of temperature. However, it is likely that frequency of this behaviour will also change in response to many factors other than stress so that its use as a welfare indicator may be inappropriate in some situations. For example, an animal may be inactive due to increased vigilance and visual interest in other animals, or the observer. A pragmatic approach that uses a variety of behavioural measures as well as environmental observations would be useful in avoiding misinterpretation of changes in levels of inactivity as being indicative of reduced welfare.

Inactive (watching observer)

The amount of time spent 'watching observer' significantly decreased following the stressor in the untrained animals. The pattern for the trained animals, although similar, fell just below the significance threshold. Exactly the same patterns appeared with respect to behaviour over time as were discussed above for inactive (all categories), and reasons for them are likely to be broadly similar.

Trained animals spent significantly more time watching the observer than untrained animals, when pre- and post-stressor data were combined. This may be due to the fact that the trained animals were regularly given food rewards by the experimenter, and the increase in inactivity (watching observer) may reflect increased vigilance associated with expected reward. The untrained animals, in

contrast, had never been given rewards by the experimenter. The explanation for the interaction between 'training' and 'time' for this behaviour is unclear.

Inactive (not watching observer)

There was no significant difference in time spent inactive (not watching observer) following the stressor for trained or untrained animals. However, trained animals spent significantly less time performing this behaviour than untrained animals. The total amount of inactivity did not differ between the two groups. Differences in amounts of inactive (not watching behaviour) are the converse of the pattern seen for inactive (watching observer) and are likely to be due to the same factor, that is trained animals spend less time ignoring the observer than do untrained animals due to the possibility of a reward.

It appears from the data presented here that the most interesting and potentially useful results are found for the combined category of inactive behaviours. This has the potential to be a useful indicator of stress and reduced welfare in the common marmoset. By contrast, little is to be gained by considering this behaviour as two separate categories, 'inactive, alert' and 'watching observer'. Any differences in the rates of these behaviours appear to be due to expectation of a reward, which may in fact mask any real effects that the experimenter is interested in. Chapters 5 and 6, which use behavioural measures in an attempt to assess welfare implications of variations in feeding predictability, therefore only use the combined category of inactivity. Data relating to the subcategories of inactive behaviour are, however, presented in the Appendix.

Locomote

There was no difference in the amount of time trained animals spent locomoting pre- and post-stressor. However, untrained animals showed a

significant increase in locomotion following the stressor. This was only evident when four sample periods were used in analysis (*i.e.* 1000h, 1200h, 1400h and 1600h). The difference became non-significant when the 1000h period was omitted. As data from the corresponding 1000h period for the trained animals were not available, it is impossible to say if the difference in significance between the two groups was due to any real difference between them, or just due to the omission of the 1000h data for the trained animals. There was no significant difference between the two groups for this behaviour, so it is possible that the missing 1000h data are responsible for the disparity between these results. This highlights the importance of collecting behavioural data immediately following the stressor, as this is when the greatest behavioural change, and hence the greatest significance in the result, is likely. Collection of longer-term data is also important in order to establish how long the effects last.

However, when data for the two groups were combined, still omitting the 1000h data, there was still a significant increase in locomotion post-stressor. This is likely to be due to the increased sample size obtained by pooling data from the two groups, and indicates that locomotion was still increased even though behavioural observations were not used from immediately following the stressor. These results suggest that, in studies with a large sample size at least, increased levels of locomotion may be a useful and relatively long lasting measure of stress and possibly reduced welfare.

This finding is supported by the results of a previous studies. Barros and co-workers (2000) found that in black tufted-eared marmosets *(Callithrix penicillata)* exposed to a taxidermised predator, locomotion decreased with moderate (2mg/kg) and high (3mg/kg) doses of the anxiolytic drug diazepam, but that a low dose (1mg/kg) had no effect when compared to the control condition (the physiological saline solution vehicle was injected without diazepam). The researchers also noted that the amount of time spent in the section of the cage nearest to the 'predator'

increased significantly at the 2mg/kg dose but not at either of the other two doses. They concluded from this that the drug had anxiolytic properties at the 2mg/kg dose, whereas the lower dose (1mg/kg) was not enough to reduce anxiety. The highest dose was thought to have a sedative effect that interfered with the anxiolytic action. The reduction in locomotion seen at the 2mg/kg dose may have been a result of the drug's anxiolytic properties rather than the sedative properties that are likely to be responsible for the decrease in locomotion seen at the higher dose. If this is the case, a reduction in anxiety is associated with a decrease in locomotion, which suggests that high levels of locomotion may indeed be an index of anxiety in the marmoset.

Smith and co-workers (1998) found locomotory behaviour to be positively correlated with urinary cortisol in Weid's black tufted-ear marmosets *(Callithrix kuhli)* when housed alone in a novel cage. Several other stressors, such as separation from conspecifics (Levine *et al*, 1993), novelty (Hennessy *et al*, 1995) and fear stimuli (Coe *et al*, 1982) have also been shown to produce increases in locomotion in primates.

The fact that increased levels of locomotion suggest increased stress in this species is interesting, as some authors aim to increase locomotion with the use of environmental enrichment in an attempt to improve welfare. Provision of manipulable and edible materials to captive orangutans *(Pongo pygmaeus)* resulted in increases in manipulation and locomotion, both of which were considered to be beneficial for welfare (Tripp, 1985). It is possible that in some situations, however, increased locomotion may be indicative of increased arousal and possibly anxiety.

It is true that many captive primates are overweight and suffer from obesity-related problems, and increased locomotion may help to alleviate this. However, it is simplistic to suggest that increasing locomotion by any particular method will automatically improve welfare. It could be the case that certain environmental

manipulations are sources of stress to animals, and may actually be detrimental to their welfare.

However, as was the case for inactivity, changes in frequency of locomotion may be in response to factors that are apparently not stressful. For example, giving an animal a larger enclosure or scatter feeding instead of putting all the food in one place so that the animal has to search for it, may result in increased locomotion. This again underlines the need for a considered and common-sense approach when interpreting changes in behaviour as indicators of welfare. Interplay between the two factors means that increases in locomotion are likely to be proportional to decreases in inactivity.

Forage

There was no significant difference in the amount of foraging following the stressor in the trained or untrained animals. There was an interaction between 'stress' and 'training' for this behaviour, with trained animals foraging more, and untrained animals foraging less, post- as compared to pre-stressor. The reasons for this are unclear. However, frequencies of this behaviour were so low throughout the study, it is possible that the effect is an anomaly of the data.

Self-scratch

There was no significant difference in amount of self-scratching following the stressor in the trained animals. However, the untrained animals showed a significant increase in self-scratching post-stressor, for both analyses (*i.e.* for the analysis of time periods 1000h, 1200h, 1400h and 1600h, and also for the analysis where the 1000h period was omitted, to match the observations made on the trained animals). When data for trained and untrained animals were pooled, there was also an overall significant increase in self-scratching post-stressor. The interaction between 'stress' and 'time' for the combined data of trained and untrained animals

indicated that the greatest increase in self-scratching occurred during the earliest observations following the stressor (*i.e.*, at 1200h), and returned almost to baseline levels by 1600h.

Self-scratching is thought to be a displacement activity in primates, which occurs during situations of tension, uncertainty, frustration, conflict and stress (Diezinger & Anderson, 1986; Maestripieri *et al*, 1992b). Behavioural studies have shown a positive correlation between amount of self-scratching and the degree to which a situation is likely to be stressful (Baker & Aureli, 1997; Pavani *et al*, 1991). In pharmacological studies, benzodiazepine anxiolytic drugs have been found to reduce the frequency of self-scratching in species of macaque (Maestripieri *et al*, 1992a; Schino *et al*, 1988) and marmoset, including the common marmoset (Barros *et al*, 2000; Cilia & Piper, 1997). Self-scratching is widely recognised as an indicator of anxiety in studies on non-human primates (Schino *et al*, 1988; Troisi *et al*, 1991).

The amount of self-scratching was significantly higher in untrained than trained animals, when pre- and post-stressor values were combined. The positive interaction between 'training' and 'stress' showed that whereas trained animals showed no difference in scratching post-stressor, there was almost a three-fold increase in self-scratching in the untrained animals following the stressor. The fact that amount of self-scratching was similar for both groups pre-stressor suggests that training animals has no effect on their pre-stressor, undisturbed behaviour. However, being exposed to training procedures may mean that these animals are less affected by stressors than their untrained counterparts, as is evidenced by the similarity between pre- and post- stressor levels of self-scratching. Unfortunately, data from the period immediately following the stressor are not available, so it is impossible to say whether trained animals are genuinely unaffected by the stressor with respect to this behaviour, or whether they merely return to baseline levels more quickly.

Scent mark

Frequency of scent marking was not significantly different in trained or untrained animals following the stressor. However, when data from both groups were pooled, and so sample size was effectively increased, there was a significant increase in this behaviour post- stressor. There was no significant difference between trained and untrained animals in the amount of scent marking observed. Nor were there any significant interactions between any of the variables (*i.e.*, 'stress', 'time period' or 'training'). The fact that pooled data did show an increase in scent marking after the stressor suggests that this behaviour may be an indicator of stress in this species, albeit a less sensitive one requiring a larger sample size to show significance than, for example, self-scratching.

Scent marking in marmosets is thought to be important in sexual communication and in the demonstration of social dominance in both sexes, and also appears to play a role in territorial behaviour (Epple, 1970). Exploration of a new or altered environment is associated with an increase in frequency of scent marking of mammals in general (see Eisenberg & Kleiman, 1972, for a review) and callitrichid primates in particular (Epple, 1970; Smith & French, 1998). Such scent marking may be due to re-establishment of territory; however, it may also be indicative of general arousal.

Epple (1978) found that scent marking in saddle-backed tamarins *(Saguinus fuscicollis)* increased significantly during aggressive interactions with unfamiliar conspecifics. Scent marking in golden lion tamarins *(Leontopithecus rosalia)* followed situations of high arousal such as chasing and wrestling between group members, and could also be stimulated by human disturbance (Mack & Kleiman, 1978). The researchers concluded that

> 'scent-marking appears to occur frequently when lion tamarins are highly aroused' (p. 186).

Similarly, the increased scent marking that regularly precedes and follows copulation in common marmosets, previously assumed to be due to the role of scent marking in sexual communication (Epple, 1970) may be at least partly attributable to the increased arousal of the situation. Odours produced by scent marking may however be arousing in themselves. Eisenberg and Kleiman (1972), state that

> 'some species-specific odors may act soley to arouse the recipient; of course, those odours which contain information about species, sex, mood, etc, will also have arousal properties' (p. 22).

Thus there may be a circular phenomenon occurring, with heightened arousal leading to an increase in the frequency of scent marking, which in turn functions to increase arousal, and so on.

Some researchers go further and consider scent marking to be an anxiety-related behaviour in the marmoset, as it is affected by various classes of anxiolytic drugs (Cilia & Piper, 1997; Costall *et al*, 1988). In an experiment using a taxidermised predator to elicit fear and anxiety, scent marking in black tufted-eared marmosets was found to disappear after administration of moderate and high doses of the anxiolytic benzodiazepine diazepam (Barros *et al*, 2000). However, no statistical significance was reached in comparison with the control group. The researchers suggest that this could be due to the low baseline levels or the small sample size used in the study, and consider scent marking to be a possible index of emotionality in the marmoset.

Another interesting finding from the present study was that there was no overall difference in the amount of scent marking shown by trained and untrained animals. This is surprising, as the trained animals had been trained to perform this behaviour in order to receive a prized reward. It might be expected that they would therefore scent mark more than the untrained animals, in response to the presence of the observer in front of the cage. It is, however, possible that the untrained animals scent marked more both in response to the stressor and to the presence of the

observer. Such increases may have been cancelled out by the increased levels of scent marking shown by the positively reinforced trained animals.

Vocalise

There was no significant difference in the amount of vocalisation following the stressor for trained animals, untained animals, or for the pooled data for both groups. Nor was there any significant interaction between the variables for this behaviour. Vocalisation of domestic cattle *(Bos taurus)* has been described as

> 'a subjective commentary, by an individual, on its own internal state' (Watts & Stookey, 2000, p.16).

These researchers propose that the study of vocalisations may be a useful method for investigating the physical and psychological functioning, and therefore the welfare, of these animals. Likewise, it has been suggested that certain vocalisations in François langurs *(Presbytis françoisi)* may be associated with stress (Krishnamurthy, 1994). Vocalisations in rhesus macaques *(Macaca mulatta)* are also thought to vary with housing and treatment conditions (Mulligan *et al*, 1994). Vocalisations may be monitored non-invasively and remotely, and may provide a continuous means of tracking changes in an animal's emotional state contingent on varying treatment and environmental conditions (Mulligan *et al*, 1994).

Many different types of marmoset calls have been categorised; for example, Stevenson and Poole (1976) described six types of vocalisation, plus various combinations of these. Epple (1968) described a range of vocalisations produced by common marmosets in various situations, such as isolation from other members of the group and exposure to predators. The vocalisations described included contact, mobbing and warning calls, as well as those associated with submission, aggression, anger and fright. Different types of contact calls were given which were thought to indicate comfort and satisfaction or distress. Marmosets also produced alarm calls in response to unexpected movements (Pook, 1978). Jones (1997) found that vocal

behaviour of common marmosets showed pronounced changes over the course of pair formation. She went on to suggest that information present in vocalisations could be used to monitor responses to social changes in this species.

There are two possible reasons that no significant differences were found between conditions, in the present study, for vocalisations. Firstly, recorded frequencies of this behaviour were extremely low throughout the observation periods. No discrimination was made between types of call in data collection, due to difficulty in distinguishing the calls. There may have been differences in *type* of vocalisation rather than in overall *frequency* of vocalisation, but it was impossible to be able to discern such differences from the data. Secondly, the room in which each pair was housed contained around 60 other marmosets, and noise from these other animals made it impossible in many cases to tell which marmoset was making each sound. Therefore, vocalisations were only recorded if the individual was actually *seen* as well as heard to vocalise; consequently it is likely that many instances were missed, for example if the animal had its head turned away as it made the sound. Additionally, marmosets make some vocalisations with the mouth closed (Epple, 1968), and it was not possible for these to be recorded in this study. Further studies are necessary to investigate marmoset vocalisations made in different contexts and from these to ascertain whether any particular forms of vocalisation are associated with stressful situations. Vocalisation data collected within the constraints described here appear to have little utility as practical welfare indicators in this species. This measure was therefore omitted from the main analyses in Chapters 5 and 6, although the data are presented in the Appendix.

Time spent in nest box

There was no significant difference in the amount of time the trained or untrained animals spent in the nest box following the stressor. Findings from other studies suggest that time spent in the nest box may be a useful measure of the

perceived unpleasantness of a stressor. Pedersen and Jeppesen (1993) found that when blue foxes *(Alopex lagopus)* were disturbed they would retreat into their nest box. They proposed that the nest box served as a hiding place in which the animals could retreat from disturbing external stimulation. Silver fox *(Vulpes vulpes)* vixens with access to a nest box also showed lower base levels of cortisol, less fear towards humans and more exploration during an open-field test than those without a nest box (Jeppesen & Pedersen, 1991).

The marmosets in this study, however, did not use the nest box more following the stressor. This may be due to the fact that, as part of the stressor, they had been captured in the nest-box in order to be removed from the cage. This may have changed their perception of the nest box; rather than being a safe hiding place, it may have been perceived as a place in which they could be trapped. Further studies could be carried out, using other stressors or methods of capture, to establish whether marmosets that have not been previously trapped in the nest box spend more time in there following a stressor. It is possible that providing marmosets with nest boxes may significantly reduce their stress levels, as was seen in silver foxes (Jeppesen & Pedersen, 1991). The length of time spent in the nest box may be negatively correlated with stress, as it allows the animal to escape from an aversive situation or perceived threat. If this is the case, the method of capture employed by the MRC laboratory, although apparently quick and easy, may actually be detrimental to the welfare of the marmosets as it effectively removes the secure 'hiding place' that would otherwise be available to the animals. Once again, for this population of animals at least, the amount of time spent in the nest box appears to be of limited practical use as a welfare indicator. It is therefore not included as such in the following studies.

It should be noted that for many behaviours, there may be wide ranges of acceptable time budgets within which welfare is not compromised. The challenge

remains to be able to quantify what frequencies of each behaviour are normal and acceptable, and at what stage changes in behavioural frequency may actually represent a threat to welfare.

Effects of diurnal variation on urinary cortisol

Faecal (Raminelli *et al*, 2001; Sousa & Ziegler, 1998) and salivary (Ashley *et al*, 2001) cortisol have been found to fluctuate according to the time of day in the common marmoset. However, the relationship between circadian rhythms and urinary cortisol in this species has not been examined previously, although marked circadian variations on urinary cortisol levels have been demonstrated for Weid's black tufted-ear marmosets, another callitrichid species (Smith & French, 1997). Data concerning diurnal variations of cortisol in blood for this or any other species of callitrichid primate are unavailable. However, the squirrel monkey *(Saimiri sciureus)*, another New World primate, shows variation in plasma cortisol levels, the highest concentrations occurring at 0400h (Coe & Levine, 1995). Diurnal species tend to show a peak in plasma cortisol before the animal becomes active in the morning (McIntosh *et al*, 1981).

This study found that male marmosets did not show diurnal variation in urinary cortisol excretion. Female marmosets showed significant variation, but only when data were collapsed into two-hour time blocks. These results are supported by those from previous studies, which focused on variation in faecal cortisol. Female, but not male, common marmosets showed diurnal variation in faecal cortisol concentrations (Raminelli *et al*, 2001; Sousa & Ziegler, 1998). However, circadian patterns of urinary cortisol have been demonstrated for both male and female Weid's black tufted-ear marmosets (Smith & French, 1997). Males and females in the present study did not show significantly different levels of urinary cortisol, which contrasts with findings of previous studies. Smith and French (1997) found that female Weid's black tufted-ear marmosets showed significantly higher urinary

cortisol levels than males, a finding that was replicated by Raminelli and co-workers (2001) for faecal cortisol in common marmosets. This effect has also been reported in mouse lemurs *(Microcebus murinus)* (Perret & Predine, 1984). Salivary cortisol from dominant, but not subordinate, common marmosets also appeared to be affected by diurnal variation (Ashley *et al*, 2001).

Female marmosets here showed a peak in urinary cortisol levels at around 0900-1000h. This peak may have in fact occurred earlier, but the first sample was not collected until 0900h, about an hour after the first void sample. Unfortunately, it was not possible to collect the first void urine sample of the day from this population. The marmosets tended to stay in their nest boxes until the lights were turned on at around 0800h. The first void sample would have been produced shortly after this, and would have included an accumulation of urinary steroids produced over the sleeping period (Zeigler *et al*, 1995).

Smith and French (1997) found levels of urinary cortisol in Weid's black tufted-ear marmosets to peak at around 1000-1100h. Following this peak, there was a gradual decline for females, which reached a nadir at 1600h; for the males, there was a dramatic drop at around 1200-1300h and levels remained low for the rest of the day. The peak in faecal cortisol found by Sousa and Zeigler (1998), by contrast, was seen at around 1400-1600h. The delay in excretion of faecal cortisol, in relation to circulatory (plasma) cortisol is likely to be around 8-10 hours (Sousa & Zeigler, 1998). The peak in urinary cortisol found at 0900-1000h in the present study suggests that excretion of cortisol in urine is quicker than in faeces, possibly taking around 3-4 hours. This would concur with findings by Smith and French (1997), who found that increases in cortisol following a stressor were detectable in urine within 4 hours.

Effects of a stressor on urinary cortisol.

The marmosets in this study showed no significant differences in urinary cortisol levels in relation to the stressor, although there was a strong trend towards an increase in cortisol post-stressor, which just failed to reach significance. It is possible that with a larger sample size, a significant result may have been obtained. Other studies have found clear increases in urinary cortisol in callitrichid primates in response to a stressor. For example, female cotton-top tamarins *(Saguinus oedipus)* showed elevations in urinary cortisol when removed from the natal group and exposed to a novel male (Ziegler *et al*, 1995). Isolation in a small cage for around 11 hours produced significant increases in urinary cortisol in Weid's black tufted-ear marmosets (Smith & French, 1997).

There was a significant effect of time on urinary cortisol pre- and post-stressor, when 1000h samples at baseline and from the morning following the stressor were included in the analysis. This appears to be due to the inclusion of the 1000h data, as the corresponding analysis without these values was not significant. The 1000h values were higher than those at other times of the day, although not significantly so, and this is likely to account for the difference in overall significance. The peak in cortisol at 1000h is in agreement with findings by Smith and French (1997) and with data presented earlier in relation to circadian variation in cortisol. The elevation in cortisol post-stressor at 1000h may account for the difference in significance between this result and that discussed earlier, in which no overall significant variation was found as a result of circadian rhythms in the baseline data. However, this elevation was not sufficient to produce a significant effect of stress on urinary cortisol. Smith and French (1997) found the clearance rate of cortisol to be rapid. Although levels of urinary cortisol in Weid's black tufted-ear marmosets was significantly elevated at 1800h following the stressor, which was administered at around 0700h, the first void sample of the following day was not significantly higher than baseline.

There are several possible reasons why there was no significant increase in cortisol following the stressor in this study. The stressor used may not have been sufficiently aversive to provoke a reaction in the animals in the present study. The trained animals (the only ones from which urine was collected) showed very little behavioural change following the stressor, which supports this suggestion. Stressors used in other studies may have had a greater effect on cortisol production because they were more aversive to the animals. For example, Smith and French (1997) used isolation for 11 hours in a novel cage as a stressor, whereas in this study, animals were removed from the home cage for around 4 minutes, and for part of that time were still in contact with their cage mate.

The increased human contact and interaction that the marmosets underwent as a result of the training for urine collection may also have had a beneficial effect on their reactions to being handled and temporarily removed from the home cage. Fear responses in the stressor situation may have been lessened due to the marmosets' previous experience with human interaction, which was mainly comprised of positive reinforcement and frequent rewards. The trained animals therefore may have perceived the stressor differently to the untrained animals, which had less prior experience of positive human interaction. Psychological factors play a significant role in the stress response (Mason, 1968); changed perception of the stressor may therefore have altered behavioural and physiological reactions to it. The results of this study therefore suggest that exposing marmosets to positive human interaction may help them to better cope with routine laboratory procedures, such as being removed from the home cage and weighed.

Prior experience of positive handling affects responses to stressors in many species of animal, and taming may reduce the physiological reactivity of the HPA (review by Grandin, 1997). Positive human interaction and handling early in an animal's life may influence its subsequent behavioural development (Hemsworth *et al*, 1986), and reduce fear of humans (Day *et al*, 2001; Hemsworth & Barnett, 1992;

Tanida *et al*, 1994; 1995). Calves accustomed to petting by visitors to a university experiment station showed lower elevations in cortisol than those that had been handled less frequently (Boandle *et al*, 1989). Hand-reared deer had lower cortisol levels after restraint than free-ranging deer, although they struggled equally intensely during the restraint (Hastings *et al*, 1992). Reduced fear of humans was so marked in weanling pigs exposed to pleasant human handling that they became more difficult to handle, taking longer to exit a pen than their minimally handled counterparts (Day *et al*, 2001). The animals in these studies were all handled when young; however, the present data indicate that exposing marmosets to positive handling when adult may also have a beneficial effect on their ability to cope with stressors. Common marmosets accustomed to handling and biweekly cage transfers did not show an immediate elevation in plasma cortisol when exposed to a novel environment with an unfamiliar, opposite-sex partner (Norcross & Newman, 1999).

Animals may learn to tolerate stimuli that may initially be frightening, such as human presence, with positive rewards (Laule & Desmond, 1998). The fearsome stimulus gradually becomes less aversive and less likely to produce a stress response, with successive desensitisation to it. It is possible that the trained animals here showed a generalisation of desensitisation to other novel or unexpected stimuli. This may be especially true as the stressor used here involved human handling, and was mainly carried out by Jean McKinley, who also trained the animals. It is possible that if a different stressor had been used, such as being isolated for a lengthy period, or being put into a cage adjacent to that of an aggressive conspecific, more significant increases in cortisol and stress-related behaviours may have been seen. Additionally, had a handler unfamiliar to the animals carried out the stressor, the increase in cortisol may have been significant.

The presence of the familiar pair mate during part of, and following, the stressor may also have attenuated the behavioural and physiological response. The presence of familiar peers has been shown to reduce the impact of stressors (Coe *et*

al, 1982; Gunnar *et al*, 1980; Hennessy, 1984; Hennessy *et al*, 1979; Levine *et al*, 1993). Smith and co-workers (1998) found that when Weid's black tufted-ear marmosets were housed alone in a novel cage, there were significant increases in urinary cortisol after 24 hours and 48 hours novel-cage exposure. However, when animals were housed in the novel cage in the presence of a heterosexual pair mate, there were no significant differences in urinary cortisol across the entire 4-day period of the novel-cage housing. Behavioural reactions to the novel cage were also reduced in the presence of the companion animal. This social buffering against the effects of stressors may function to regulate HPA function in marmosets (Smith *et al*, 1998).

This study showed that the response of common marmosets to a stressor was unlike that of Weid's black tufted-ear marmosets, as the former showed no differences in urinary cortisol post-stressor, whereas the latter showed significantly elevated levels (Smith & French, 1997). However, there could be many reasons for these differences in the two species of marmoset. For example, species differences in themselves may mean that cross-species generalisations are not meaningful (Mason & Mendl, 1993). Common marmosets have been used as laboratory animals for many years, and several generations of this species have been bred in captivity for this purpose. Even if little intentional selective breeding has been carried out, a certain amount of inadvertent selection will doubtless have occurred. For example, laboratories may have preferentially bred from animals that are calmer and easier to handle; conversely, animals that are less stressed by being in the laboratory situation may have been more likely to breed and to raise viable offspring. Therefore, it is possible that common marmosets currently held in laboratories such as the MRC have adapted over generations to be better able to cope with laboratory procedures than animals that have not been held in captivity for as long, such as Weid's black tufted-ear marmosets.

Additionally, stressors in the two studies were administered at different times (0700h and 0930h); the cortisol response to a stressor has been shown to differ depending on the point in the circadian rhythm that the stressor was given (Thuma *et al*, 1995), although other research has found this not to be the case (Bradbury *et al*, 1991). Finally, as discussed above, the variation in the severity and duration of the stressors is likely to have had an effect on the results of the two studies.

4.5 SUMMARY AND CONCLUSIONS

Common marmosets were trained using positive reinforcement techniques over a period of around six weeks to provide urine samples on request. Females showed diurnal variation in the levels of excreted urinary cortisol, with a peak at around 0900-1000h, whereas levels in males were not significantly affected by circadian rhythms. Trained marmosets showed few behavioural changes following exposure to a mild stressor; the only behaviour that was significantly affected by the stressor was inactivity, which was reduced. However, marmosets that were untrained and had been exposed to minimal human interaction showed significant increases in amounts of self-scratching and locomotion, and reductions in inactivity, after being exposed to a similar stressor. When data were pooled from the trained and untrained animals the amount of scent marking was also found to increase significantly, post-stressor. Similar changes in these behaviours may be useful non-invasive measures of stress in this species, although a cautious and pragmatic approach is necessary in order to avoid misinterpretation.

The differences in behavioural reactivity shown by trained and untrained animals when exposed to the stressor suggest that the process of training marmosets may make them less affected by stressful situations, and therefore better able to cope with routine procedures in the laboratory. The trained animals (the only ones from which it was possible to collect urine) did not show elevations in urinary

cortisol in response to the stressors. This further supports the observation that they were relatively unaffected by the mild stressor, possibly as a result of the positive human interaction that they had experienced.

Chapter 5

Effects of reliability of feeding-related signals on the behaviour and welfare of common marmosets

5.1 INTRODUCTION

Feeding is an event that is likely to be of great positive significance in the day of a captive animal. Temporally predictable feeding schedules are thought to be associated with increases in stereotypies (Carlstead, 1998; Krishnamurthy, 1994), aggression (Wasserman & Cruikshank, 1993) as well as inactivity and coprophagy (Bloomsmith & Lambert, 1995); all these behavioural changes have been interpreted as being indicative of decreased welfare. Bloomsmith and Lambert (1995) found that feeding chimpanzees *(Pan troglodytes)* on an unpredictable temporal schedule led to an increase in species-appropriate behaviour, suggesting improved welfare. These researchers concluded that, in the absence of control, predictability may be more stressful than unpredictability.

However, the chimpanzees in this study (Bloomsmith & Lambert, 1995) did receive information regarding the onset of feeding. The four groups in the study were fed by keepers standing at the roof level of the enclosures. Although each group received food separately rather than all of them being fed simultaneously, keepers bringing out food were visible to some members of the colony, who typically responded with loud food vocalisations. All chimpanzees therefore received signals notifying the arrival of each meal, whether or not they were the intended recipients. The animals receiving their food on an unpredictable temporal schedule therefore received a signal that the arrival of food was imminent. Two types of predictability may therefore be identified as factors in the outcome of this study - temporal predictability and signalled predictability.

Carlstead (1986) manipulated the predictability of feeding by changing the reliability of signals (in the form of a bell) announcing the arrival of food, delivered on a variable temporal schedule, to pigs. Low signalled predictability was found to be associated with frustration, which led to aggression and increased competition for food. Frustration-induced aggression has been observed in pigeons (Azrin et al, 1966) domestic hens (Duncan & Wood-Gush, 1971), rats (Davis & Donenfield, 1967; Thompson & Bloom, 1966), pigs (Dantzer & Mormede, 1980) and squirrel monkeys (Hutchinson *et al*, 1968).

In the captive situation, there will always be certain external signals associated with feeding, such as the sound of food preparation, doors being unlocked or other animals being fed. These signals may not always be reliable, especially in situations where there are many animals and therefore many feeding-related signals. Unreliable signals may lead to aversive primary frustration reactions (Amsel, 1958), but it is unrealistic to expect these signals to be eliminated. In these situations, it may be useful for animals to learn to associate a unique noise, such as a buzzer or bell, with feeding. This sound would only be heard prior to feeding, and may help to extinguish previously learned signal associations as described above, which may not be reliable. It may be possible, using such a method, to feed on an unpredictable temporal schedule and derive benefits such as those seen by Bloomsmith and Lambert (1995), but without the negative consequences observed by Carlstead (1986). It has been suggested that, for optimal welfare, predictability of environmental events should be of an intermediate value (Novak & Drewson, 1989; Wiepkema & Koolhaas, 1993); temporally unpredictable feeding preceded by a reliable signal may provide such an intermediate value.

The present study investigated the effects of signalled predictability using food deliveries of varying predictability. All the food presentations were to an extent temporally unpredictable, as they occurred at varying times during the data collection period. However, the predictability of the food delivery was manipulated

in one of three ways. The most predictable food, given in the Condition A, was preceded by a signal, consisting of a short electronic tone. This was sounded exactly one minute before the food was delivered. In Condition B, the food was delivered in the absence of a signal. Here, the animals would have had cues from the presence of the observer that food would be delivered, but they had no signals to indicate exactly when this would occur. Animals in Condition C were treated the same as those in Condition A, except that they were given food on only 50% of occasions. The signal was therefore an unreliable predictor to the animals regarding the arrival of food. This may be analogous to the real-life husbandry situation where animals may hear food being prepared or transported but this food may or may not be delivered to them – rather, it may be intended for other individuals.

The first condition represented an intermediate predictability of feeding, and was expected to be associated with low levels of stress-related behaviours. The second condition represented a more unpredictable feed; I expected stress-related behaviours to be intermediate between those seen in conditions one and two. The third condition represented an unpredictable food delivery with the additional variable of an unreliable signal; I predicted that more stress-related behaviours would occur in this condition. A fourth condition was included as a control; here, neither signal nor food was given.

5.2 METHOD

5.2.1 Study animals and housing

Study animals were 48 common marmosets, 24 males and 24 females, housed at the Medical Research Council (MRC) Human Reproductive Sciences Unit, Bush Estate, Edinburgh. The mean age of the animals was 935.54 days (± S. E. 88.08 days). Mean ages of animals in each of the four experimental groups (n=12 in each group) were not significantly different from each other ($F_{3,44}$=0.49; p=0.69; n.s.) (see Table 5.1). For the main section of the study, a between-subjects

design was used, with study animals divided into four groups of six pairs, and each group exposed to one experimental condition. Each experimental group contained two mixed-sex pairs, two male pairs and two female pairs. Study animals were matched across conditions for age. See Table 5.1 for the mean ages of animals in each condition, and Chapter 3 for details of housing and husbandry routines.

Table 5.1 Mean ages of animals ± Standard Error (S.E.) in each experimental condition at the start of the study (01/09/00)

Condition	Mean Age (days)	S.E. of age (days)
A	816.92	144.46
B	986.58	187.88
C	1085.00	207.87
D	853.67	158.50

5.2.2 Experimental Design

Initially, data were collected to ascertain whether behaviour would change within sessions simply due to the presence of the observer – for example, it was thought that it may have taken a few minutes for the animals to become habituated to the presence of the observer in front of the cage. Behavioural data were collected for each of the 24 pairs for 10 minutes, and analysed to see whether frequencies of any of the behaviours changed over the 10 minute samples.

Predictability of food delivery was effected by means of a signal, which consisted of a short electronic tone that was different for each pair of study animals. The reliability of the signal, and hence the predictability of feeding, was experimentally manipulated. In the first condition, Condition A, the signal was reliable, and hence the food delivery totally predictable. In this condition, the signal was sounded between two and a half and five minutes after the start of the session, and was always followed one minute later by the delivery of the food.

The food, consisting of small pieces of marshmallow, was dropped by the observer into empty camera film cases attached by cable-ties to the inside of the

front of the cage (see Plate 13). Two film cases were attached to each cage, on opposite sides of the front mesh, and one piece of food put in each, to try to ensure that both animals were fed on every appropriate trial. Data were not collected relating to this, but the strategy was successful on most trials. This method was used as it was thought that the animals' having to take the food directly from the observer's hand might confuse appetitive and aversive stimuli. The procedure of food delivery took less than five seconds, and inadvertent signals, such as rummaging in the pocket for the food, were minimised as far as was possible.

In Condition B, no signal was sounded, but food was given on every trial. This food was delivered between three and a half and six minutes after the start of the session, and the timings of feedings were yoked to those in Condition A. Presentation of the food in one trial provided no information about the timing of feeding in subsequent trials, because different temporal schedules of food delivery were used each time. In Condition B, there was some indication to the animals that a delivery of food was imminent, as the presence of the observer was associated with this on every trial, but the timing of the feed was less predictable than in Condition A.

In Condition C, the same procedure as in Condition A was used, and once again, the timings of food deliveries were yoked to those in Condition A. However, in this condition, a piece of food was delivered on only 50% of occasions following the signal. The signal was therefore an unreliable predictor to the animals regarding the arrival of food. This may be analogous to the real-life husbandry situation where animals may hear food being prepared or transported but this food may or may not be delivered to them. Condition D was the control condition; behavioural observations were made in the absence of signal and food. The four experimental conditions are summarised in Table 5.2.

Signals were given at one of six times during the period of data collection; 2 minutes 30 seconds, 3 minutes, 3 minutes 30 seconds, 4 minutes, 4 minutes 30 seconds or 5 minutes after the start of observation. Pairs in all three experimental

Plate 13 Marmosets investigating film case, into which a piece of marshmallow has been dropped

Table 5.2 Experimental conditions used in the study

Condition	Experimental manipulation	Overall Predictability
A	*Reliable signal* During each trial, animals were given an auditory signal, followed 1 minute later by food	High
B	*No signal* Animals were given food without prior exposure to a signal	Moderate
C	*Unreliable signal* Animals received an auditory signal on each trial; on 50% of occasions, food was given 1 minute later	Low
D	*Control* Animals received neither a signal nor food	X

conditions were assigned one of the six signal and food delivery timings for each trial, following this framework, whether or not a signal or food was given in each case. The timings were randomly assigned, with each pair receiving each one twice during the study period. The food was given 1 minute after the signal; therefore, food was given 3 minutes 30 seconds, 4 minutes, 4 minutes 30 seconds, 5 minutes, 5 minutes 30 seconds or 6 minutes after the start of observation. This was to ensure that food would be *temporally* unpredictable in all cases; the factor that would differ between conditions would be the presence and reliability of the signal, and therefore the *signalled* predictability.

Pairs in Conditions B and C were yoked to pairs in Condition A, and matched across conditions regarding the timing of signal and food delivery. Each data collection session lasted for 8 minutes, which ensured that each pair was exposed to the observer for the same amount of time. This was to ensure that predictability of food was not confounded by the animals becoming more habituated to the observer. However, only data from the 2-minute period immediately preceding the signal (referred to in the analysis as Signal Period 1), the 1-minute

period between the signal and food delivery (Signal Period 2), and the 2-minute period immediately following the food delivery (Signal Period 3) were used in the analysis (See Figure 5.1). Behavioural data in these three signal periods were compared in the analysis.

For the purposes of data analysis, the control condition was assigned the same 'signal and food delivery' timings, and the corresponding five minutes from each session analysed, so that matched control data could be used as a comparison. It was thought that changes in behaviour might develop as the study progressed, and I did not know how many trials would be necessary before the unconditioned stimulus (the food) became associated with the conditioned stimulus (the signal). In order to look at this factor, the data were split into three chronological periods, which were compared. The first four trials together were categorised as Trial Period 1; the second four and third four trials respectively made up Trial Periods 2 and 3 (see Table 5.3 for a summary of the variables under investigation).

Figure 5.1 Signal periods used in the study

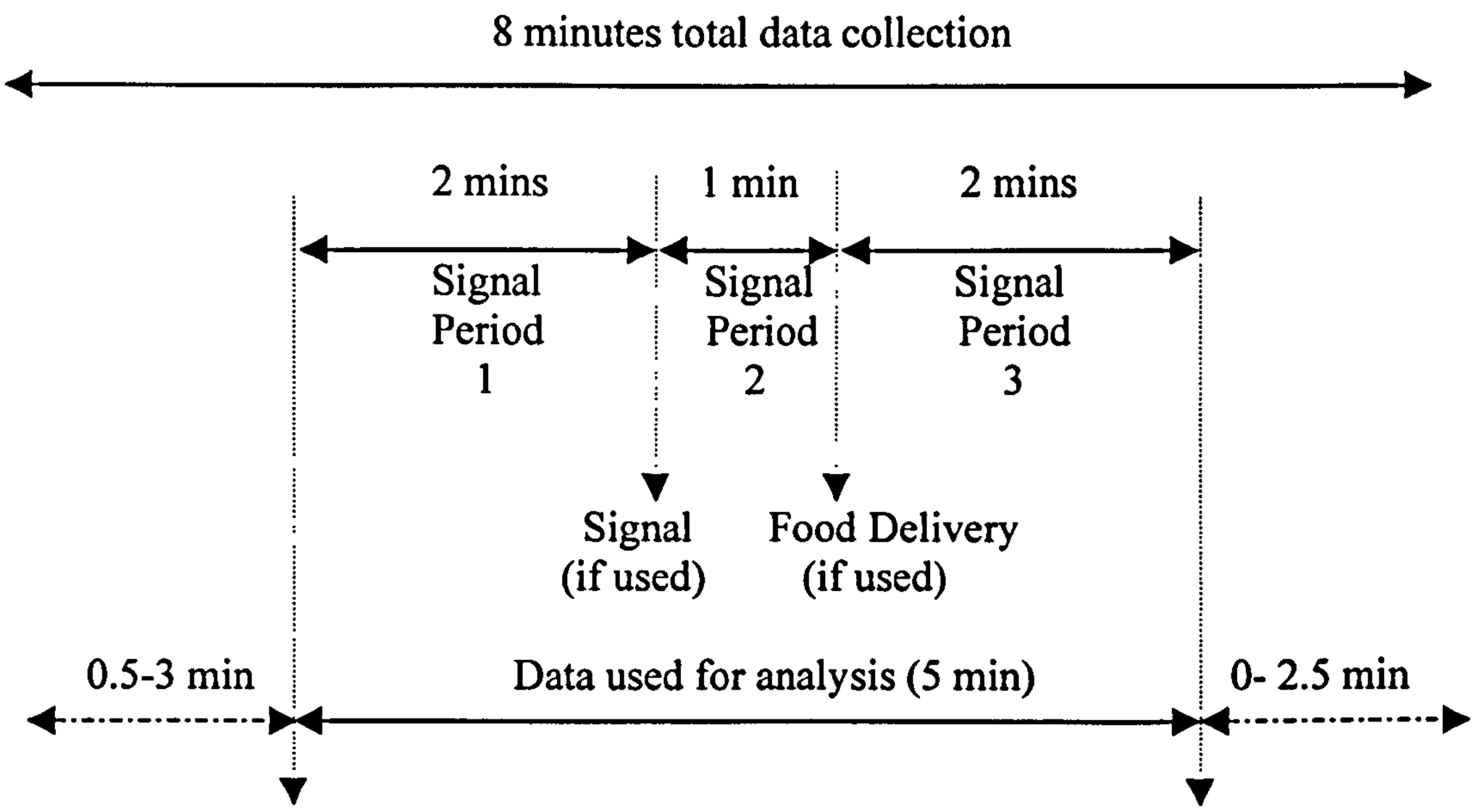


Table 5.3 Variables in the study, and number of levels within each

Variable	Within/between subjects	No. of levels	Levels
Signalled Predictability	Between-subjects	4	Condition A: Reliable signal Condition B: No signal Condition C: Unreliable signal Condition D: Control
Trial Period	Within-subjects	3	Trial Period 1: Sessions 1-4 Trial Period 2: Sessions 5-8 Trial Period 3: Sessions 9-12
Signal Period	Within-subjects	3	Signal Period 1: Pre-signal Signal Period 2: Post-signal, pre-food Signal Period 3: Post-food

5.2.3 Data collection

Data were collected on a palm-top computer, using THE OBSERVER 3.0 software. Instantaneous scan sampling was used, with an interval of 15 seconds between scans. The behaviours recorded were mutually exclusive and included: 'inactive', 'locomote', 'self-scratch', 'scent mark', 'vocalise' and 'forage', as well as an 'other' category (see Chapter 3, page 89, for behavioural definitions). As vocalisation data were not thought to be reliable welfare indicators for this population, analyses of these are not included in this Chapter. They are presented instead in Appendix 1, along with the two sub-categories of inactive behaviour 'inactive (watching observer)' and 'inactive, alert', which also, in hindsight, were not thought to be particularly relevant to the assessment of welfare (see Chapter 4 for rationale). Data relating to nest-box use were not collected for this study, as this was another measure that was not considered a reliable welfare indicator for this population.

Twelve trials were carried out for each pair, with the interval between trials being between two and four days. A total of 2304 minutes of data was collected, of which 1440 minutes were included in the analysis. Trials were evenly balanced

between morning (1100 - 1230h) and afternoon (1330 - 1500h) sessions. Observations took place over a six-week period, during September and October 2000.

5.2.4 Statistical Analysis

The behaviour of each animal was likely to have been influenced by that of its cage mate, and so data from each individual could not be treated as independent. For this reason, a single mean was calculated from the data of both animals; each pair was effectively treated as one individual in the analysis. Data were found to be normally distributed throughout and hence parametric tests were used.

The initial investigation was concerned with whether behaviour changed over the course of observation periods, without intervention from the observer. The ten minutes of data collected were split into five two-minute periods, and linear regression analyses carried out to see whether there was an increase or decrease in any of the behaviours over the observation period.

For the main part of the study, a three-factor mixed analysis of variance (ANOVA) was used to determine overall differences associated with the study variables. Interactions between the three types of experimental variable were also analysed using the three-factor mixed ANOVA. As the three signal periods were of unequal lengths, counts of each behaviour could not be used in the analysis. Therefore, frequencies of each behaviour were converted to percentages of total sample points for the purposes of the analysis.

Where significant results were obtained for the between-subjects factor of Signalled Predictability, post-hoc Tukey tests were used to compare differences in behaviour between each condition. In those cases where significant results had been obtained for the within-subjects factor of Trial Period, linear trend tests were applied to look for general increases or decreases in behaviour over the time-course of the study. This test was used only in this particular instance, as I had

hypothesised that any behavioural changes that might occur would increase or decrease, in a linear fashion, over time. Where significant results were obtained for the within-subjects factor of Signal Period, post-hoc *t*-tests, with the appropriate Bonferroni correction to guard against Type I errors, were used to compare differences in behaviour in each of the three periods.

5.3 RESULTS

Changes in behaviour due to habituation to observer

Results of linear regression analyses were all non-significant (see Table 5.4); there was no increase or decrease in frequency of any of the behaviours over a ten-minute observation period when no experimental interventions were made by the observer.

Table 5.4 Results of linear regression analyses for all behaviours in initial study to measure habituation to observer

Behaviour	F	p
Inactive	0.20	0.66
Locomote	0.35	0.55
Self-scratch	0.17	0.68
Scent mark	1.42	0.24
Forage	0.13	0.72

All d.f.= 1,118

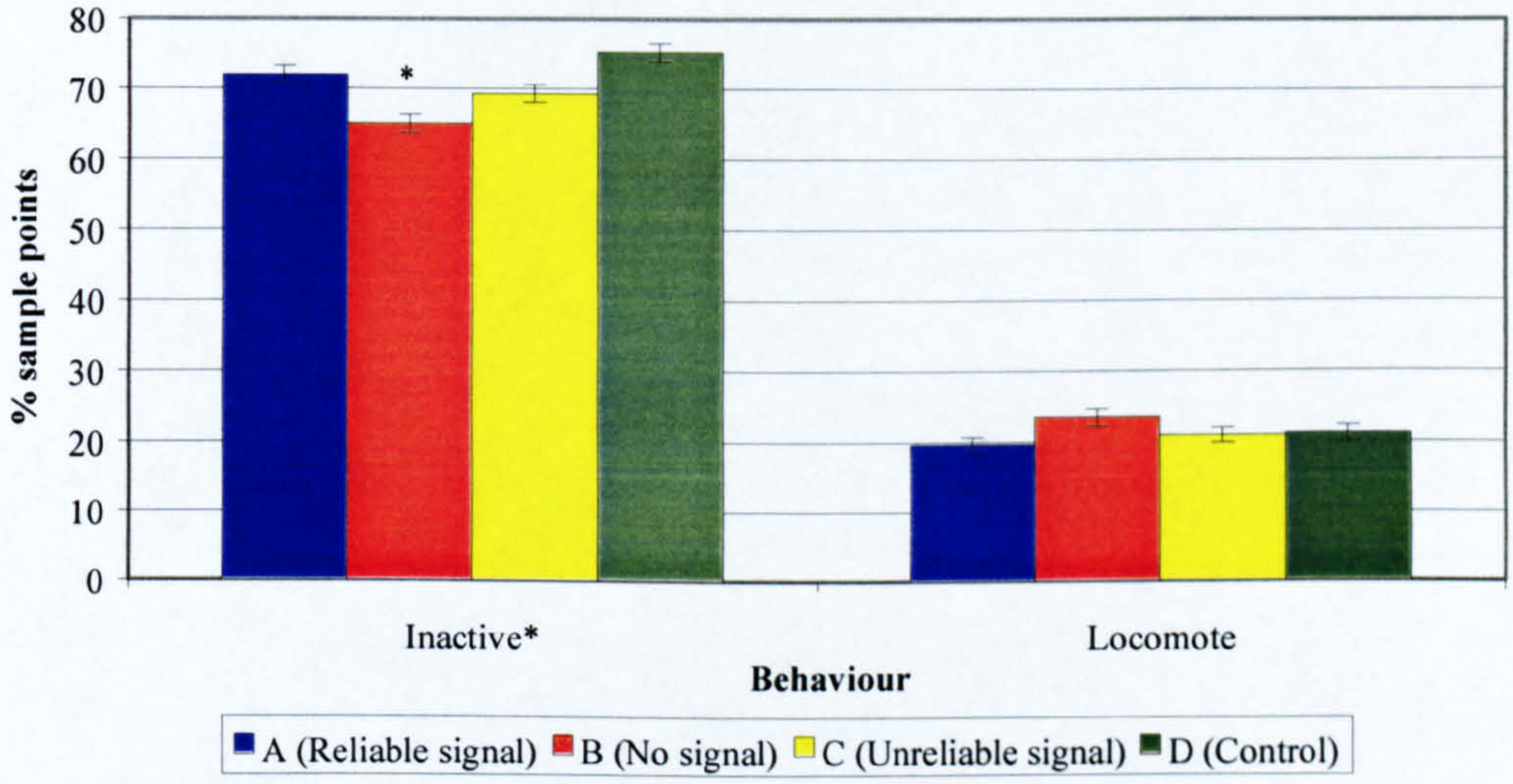
Effects of food deliveries of varying predictability on behaviour

The effects of each main factor ('Signalled Predictability', 'Trial Period' and 'Signal Period') are initially considered separately. Interactions between the variables follow these results.

5.3.1 Effects of Signalled Predictability of food delivery on behaviour

Each behavioural category is presented separately. Where a significant main effect was found, results of post-hoc Tukey tests are given.

Figure 5.2 Percentage sample points spent inactive and locomoting in the four study conditions (bars represent Standard Errors)



*<0.05; **<0.01; ***<0.001
Asterisks by x-axis labels indicate significance of ANOVAs
Asterisks at top of bars indicate significance of differences between respective condition and control (Condition D)

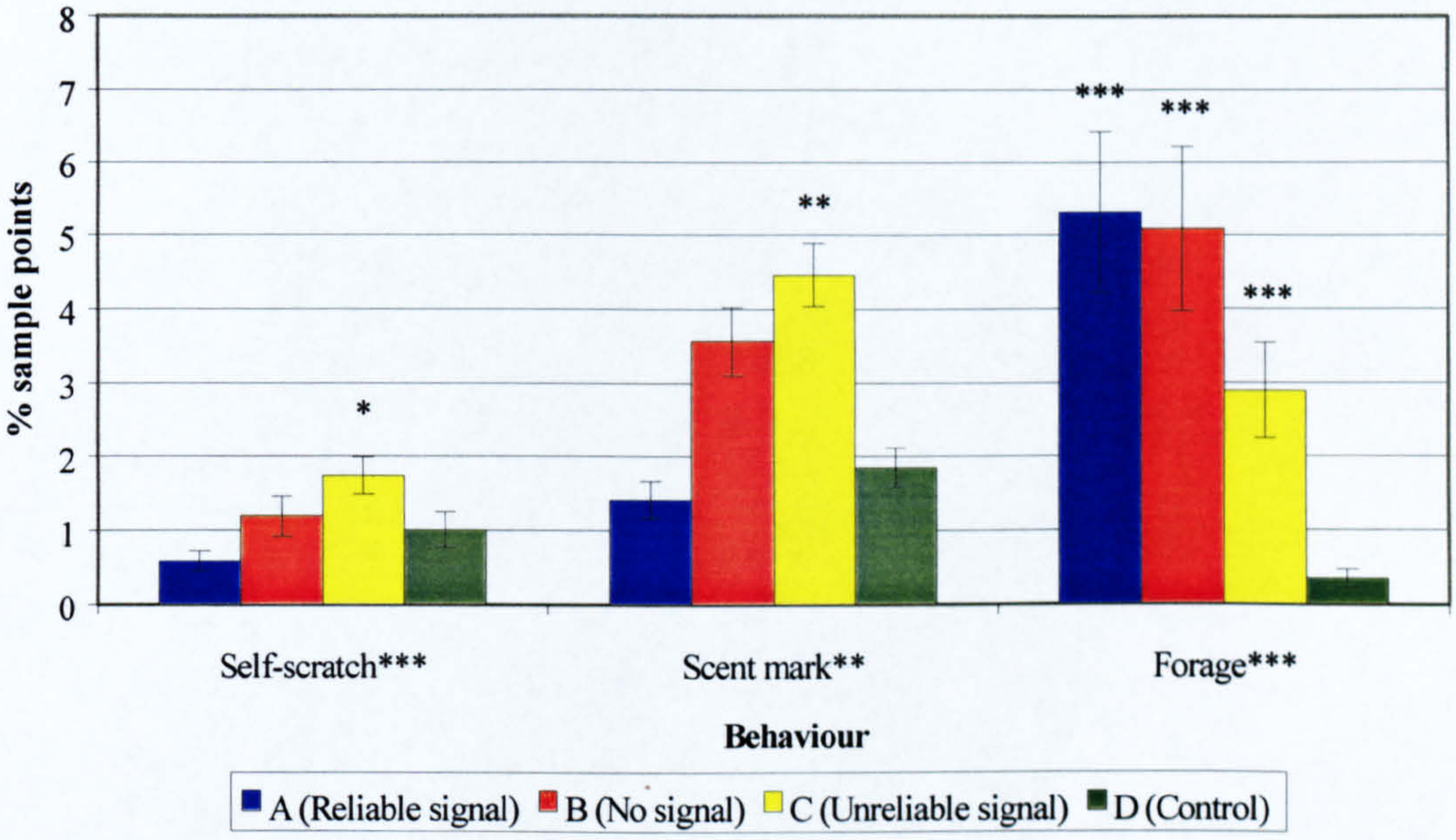
Inactive

There was a significant effect of predictability on the amount of inactivity shown (see Figure 5.2 and Table 5.5). Although pairs in both Conditions B and C showed less total inactivity than the control condition, the only significant differences between the conditions lay between Condition B and the Control (see Table 5.6).

Locomote

There was no significant effect of predictability on this behaviour (see Figure 5.2 and Table 5.5).

Figure 5.3 – Percentage sample points spent scratching, scent marking and foraging in the four study conditions (bars represent Standard Errors)



*<0.05; **<0.01; ***<0.001
Asterisks by x-axis labels indicate significance of ANOVAs
Asterisks at top of bars indicate significance of differences between respective condition and control (Condition D)

Self-scratch

There was a significant effect of predictability on this behaviour (see Figure 5.3 and Table 5.5). Animals in Condition C showed significantly higher levels of this behaviour than those in the control condition. Additionally, levels in Condition A were significantly lower than in Condition C (see Table 5.6).

Scent mark

There was a significant effect of predictability (see Figure 5.3 and Table 5.5). Scent marking was greater in Condition C than in the control condition. Additionally, levels in Condition A were significantly less than in Conditions B and C (see Table 5.6).

Forage

There was a significant effect of predictability (see Figure 5.3 and Table 5.5). Levels of foraging in Conditions A, B and C were all significantly greater than in

the control condition. Additionally, levels in Condition A were greater than those in Condition C, and Condition B greater than Condition C. The only conditions that were *not* significantly different for this behaviour were Condition A and Condition B (see Table 5.6).

Table 5.5 Results of ANOVAs for effects of signalled predictability of food delivery on behaviour

Behaviour	**F**	**p**
Inactive	4.50	<0.05*
Locomote	0.80	0.51
Self-scratch	9.61	<0.001***
Scent mark	9.04	<0.01**
Forage	63.01	<0.001***

All d.f. = 3,20

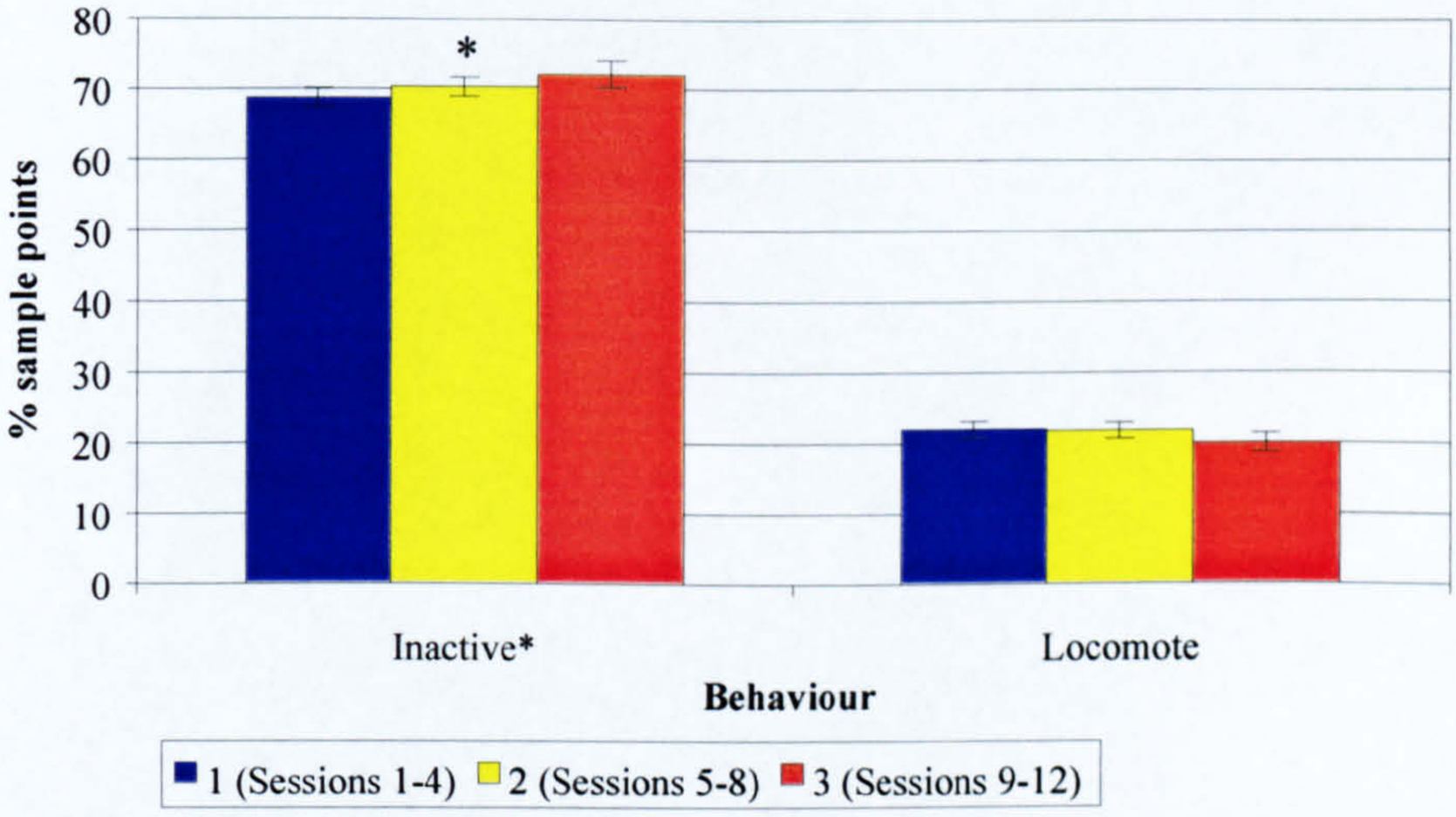
Table 5.6 Results of post-hoc Tukey tests for effects of signalled predictability of food delivery on behaviour (only behaviours showing a significant main effect of 'signalled predictability' are included)

Behaviour	Comparison between conditions	Mean Difference	S.E.	p
Inactive	A-B	6.92	2.87	0.11
	A-C	2.69	2.87	0.79
	A-D	3.24	2.87	0.68
	B-C	4.22	2.87	0.47
	B-D	10.16	2.87	<0.05*
	C-D	5.93	2.87	0.20
Self-scratch	A-B	0.61	0.22	0.06
	A-C	1.16	0.22	<0.001***
	A-D	0.43	0.22	0.23
	B-C	0.55	0.22	0.09
	B-D	0.17	0.22	0.86
	C-D	0.72	0.22	<0.05*
Scent mark	A-B	2.14	0.67	<0.05*
	A-C	3.04	0.67	<0.01**
	A-D	0.43	0.67	0.92
	B-C	0.90	0.67	0.55
	B-D	1.71	0.67	0.08
	C-D	2.60	0.67	<0.01**
Forage	A-B	0.23	0.41	0.94
	A-C	2.43	0.41	<0.001***
	A-D	4.98	0.41	<0.001***
	B-C	2.20	0.41	<0.001***
	B-D	4.75	0.41	<0.001***
	C-D	2.55	0.41	<0.001***

5.3.2 Effects of Trial Period on behaviour

Each behavioural category is again presented separately. Where a significant main effect was found, results of linear trend tests are given.

Figure 5.4 Percentage sample points spent inactive and in locomotion in the three stages of the study (bars represent Standard Errors)



*<0.05; **<0.01; ***<0.001
Asterisks by x-axis labels indicate significance of ANOVAs
Asterisks above centre bars indicate significance of linear relationship between trial period and levels of respective behaviour

Inactive

There was a significant effect of trial period on this behaviour (see Figure 5.4 and Table 5.7). Linear trend tests showed that there was a significant increase in this behaviour as trial period progressed (see Table 5.8).

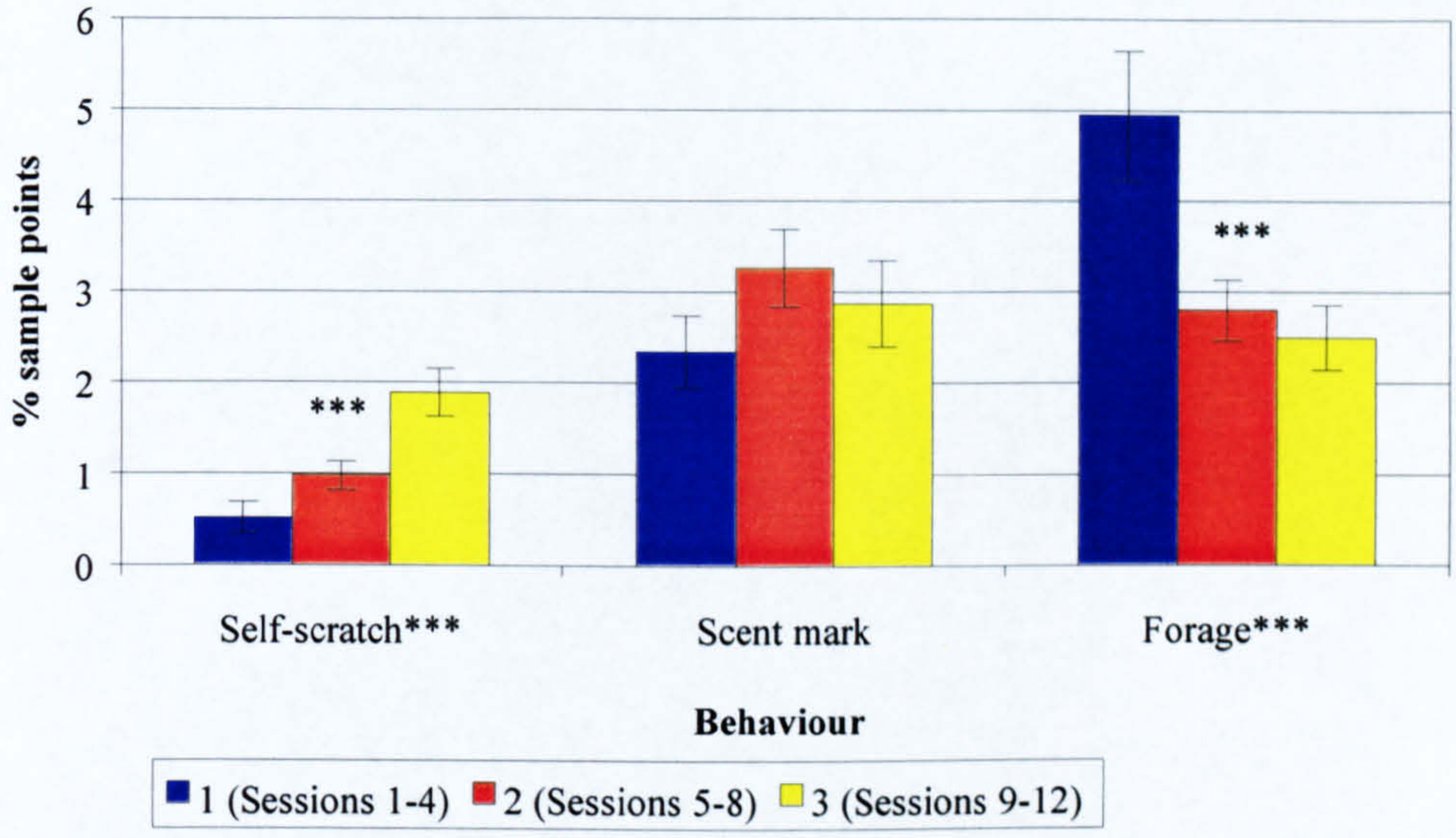
Locomote

There was no significant effect of trial period on this behaviour (see Figure 5.4 and Table 5.7).

Self-scratching

There was a significant effect of trial period (see Figure 5.5 and Table 5.7). There was a significant linear relationship between trial period and level of this behaviour; levels of scratching increased significantly as trials progressed (see Table 5.8).

Figure 5.5 - Percentage sample points spent scratching, scent marking and foraging in the three stages of the study (bars represent Standard Errors)



*<0.05; **<0.01; ***<0.001
Asterisks by x-axis labels indicate significance of ANOVAs
Asterisks above centre bars indicate significance of linear relationship between trial period and levels of respective behaviour

Scent-marking

There was no significant effect of trial period on this behaviour (see Figure 5.5 and Table 5.7).

Foraging

There was a significant effect of trial period (see Figure 5.5 and Table 5.7). There was a significant linear relationship between trial period and level of this behaviour, with levels of foraging decreasing as trials progressed (see Table 5.8).

Table 5.7 Results of ANOVAs for effects of 'Trial Period' on behaviour

Behaviour	**F**	**p**
Inactive	4.34	<0.05*
Locomote	2.50	0.10
Self-scratch	18.64	<0.001***
Scent mark	2.00	0.15
Forage	34.49	<0.001***

All d.f. = 2,40

Table 5.8 Results of linear trend tests for effects of 'Trial Period' on behaviour (only behaviours showing a significant main effect of Trial Period are included)

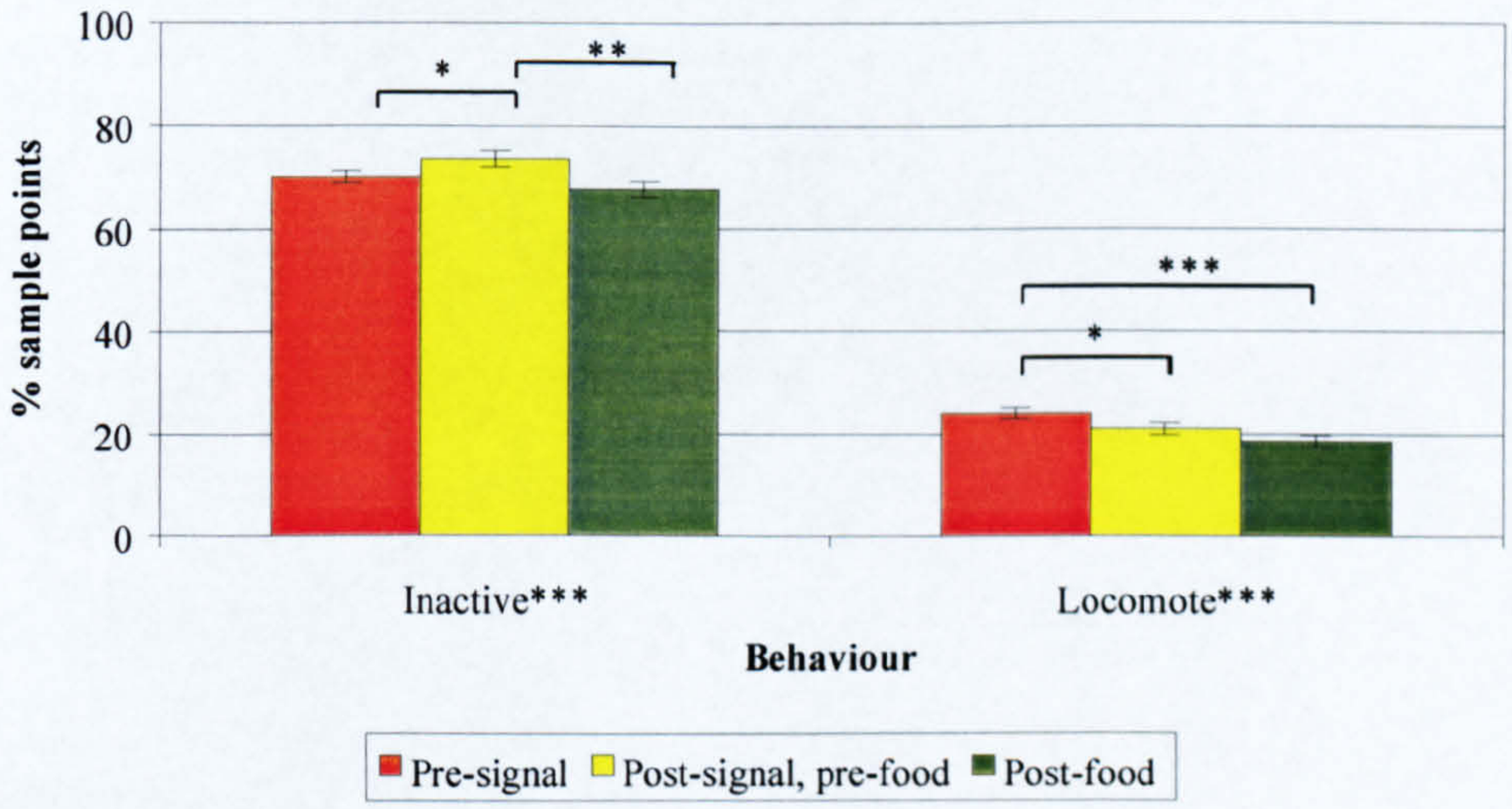
Behaviour	F	p
Inactive	8.19	<0.05*
Self-scratch	32.48	<0.001***
Forage	59.95	<0.001***

All d.f. = 1,20

5.3.3 Effects of Signal Period on behaviour

Each behaviour is again presented separately. Where a significant main effect was found, results of post-hoc pairwise *t*-tests (with the Bonferroni correction) are given.

Figure 5.6 - Percentage sample points spent inactive and in locomotion in relation to the time of signal presentation within the session (bars represent Standard Errors)



*<0.05; **<0.01; ***<0.001
Asterisks by x-axis labels indicate significance of ANOVAs
Asterisks above boxes indicate significance of post-hoc pairwise *t*-tests (following Bonferroni correction)

Inactive

There was a significant effect of signal period on this behaviour (see Figure 5.6 and Table 5.9). Levels of inactivity were significantly lower in the pre-signal period

than in the post-signal, pre-food period, and significantly higher in the post-signal, pre-food period than in the post-food period (see Table 5.10).

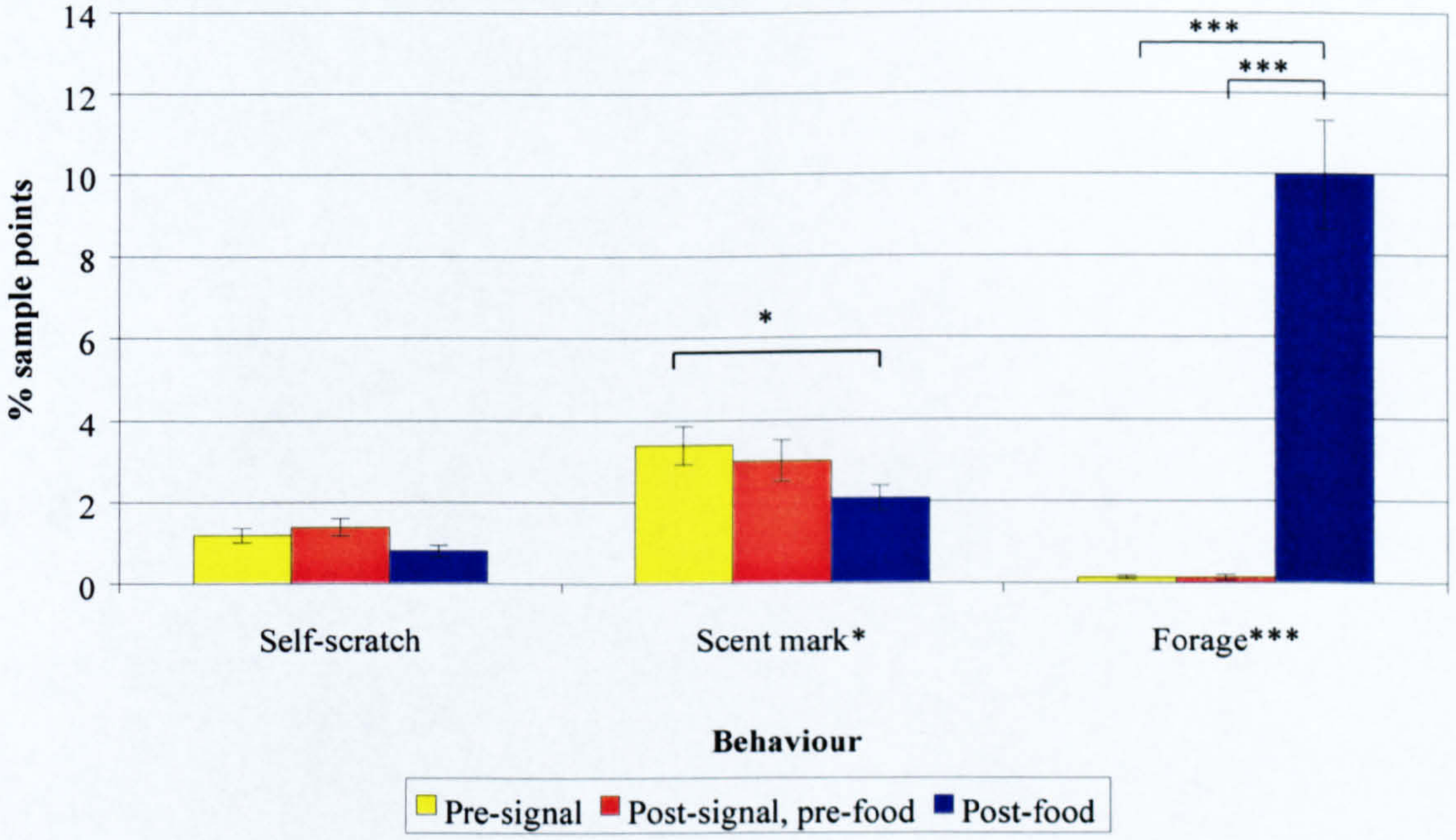
Locomote

There was a significant effect of signal period (see Figure 5.6 and Table 5.9). Levels of locomotion were significantly higher in the pre-signal period than in the post-signal, pre-food period and the post-food period (see Table 5.10).

Self-scratch

There was no significant effect of signal period on this behaviour (see Figure 5.7 and Table 5.9).

Figure 5.7 - Percentage sample points spent scratching, scent marking, vocalising and foraging in relation to time of signal presentation within the session (bars represent Standard Errors)



*<0.05; **<0.01; ***<0.001
Asterisks by x-axis labels indicate significance of ANOVAs
Asterisks above boxes indicate significance of post-hoc pairwise *t*-tests (following Bonferroni correction)

Scent mark

There was a significant effect of signal period (see Figure 5.7 and Table 5.9). Levels of scent marking were significantly higher in the pre-signal period than in the post-food period (see Table 5.10).

Forage

There was a significant effect of signal period on foraging (see Figure 5.7 and Table 5.9). Levels of foraging were significantly lower in both the pre-signal period and the post-signal, pre-food period than in the post-food period (see Table 5.10).

Table 5.9 Results of ANOVAs for effects of 'Signal Period' on behaviour

Behaviour	**F**	**p**
Inactive	14.62	<0.001***
Locomote	15.64	<0.001***
Self-scratch	2.99	0.06
Scent mark	5.02	<0.05*
Forage	518.92	<0.001***

All d.f. = 2,40

Table 5.10 Post-hoc *t*-test *t* and p values for mean percentage sample points spent in each behaviour in each 'Signal Period' (only behaviours showing a significant main effect of Signal Period are included)

Behaviour	**Signal period**	***t***	**p** (uncorrected)	**p** (following Bonferroni correction)
Inactive	1-2	2.95	<0.01**	<0.05*
	1-3	2.02	0.06	0.17
	2-3	4.22	<0.001***	0.001***
Locomote	1-2	2.94	<0.01**	<0.05*
	1-3	5.33	<0.001***	<0.001***
	2-3	2.01	0.06	0.17
Scent mark	1-2	0.80	0.43	1.00
	1-3	3.32	<0.01**	<0.01**
	2-3	1.83	0.08	0.24
Forage	1-2	0.00	1.00	1.00
	1-3	7.23	<0.001***	<0.001***
	2-3	7.20	<0.001***	<0.001***

5.3.4 Effects of interactions between the three variables

5.3.4.1 Interactions between 'Trial Period' and 'Signalled Predictability'

There were significant interactions between 'Trial Period' and 'Signalled Predictability' for 'Inactive', 'Locomote', 'Self-scratch' and 'Forage' (see Table 5.11). Significant interactions are described.

Table 5.11 Results of ANOVAs for interaction between 'Trial Period' and 'Signalled Predictability' on all behaviours

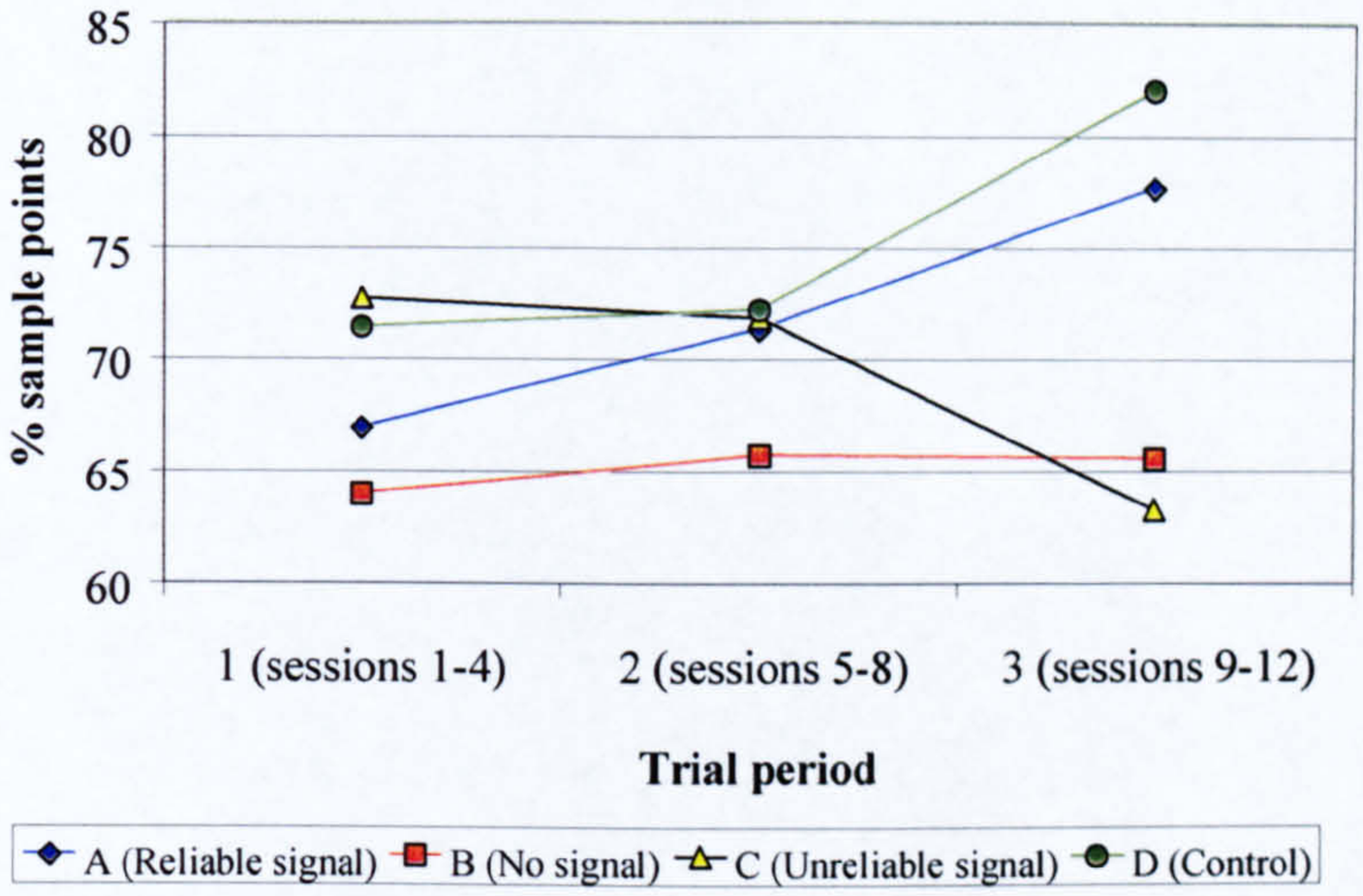
Behaviour	F	p
Inactive	10.31	<0.001***
Locomote	9.86	<0.001***
Self-scratch	5.72	<0.001***
Scent mark	0.97	0.46
Forage	5.52	<0.001***

All d.f. = 6,40

Inactive

There was a significant interaction between 'Trial Period' and 'Signalled Predictability' for this behaviour (see Figure 5.8 and Table 5.11). The amount of inactivity in Conditions A and D increased over trials, whereas that in Condition C decreased over trials. There was no apparent effect of trial on amount of inactivity in Condition B. For Trial Period 3, levels in Conditions A and D were greater than in Conditions B and C.

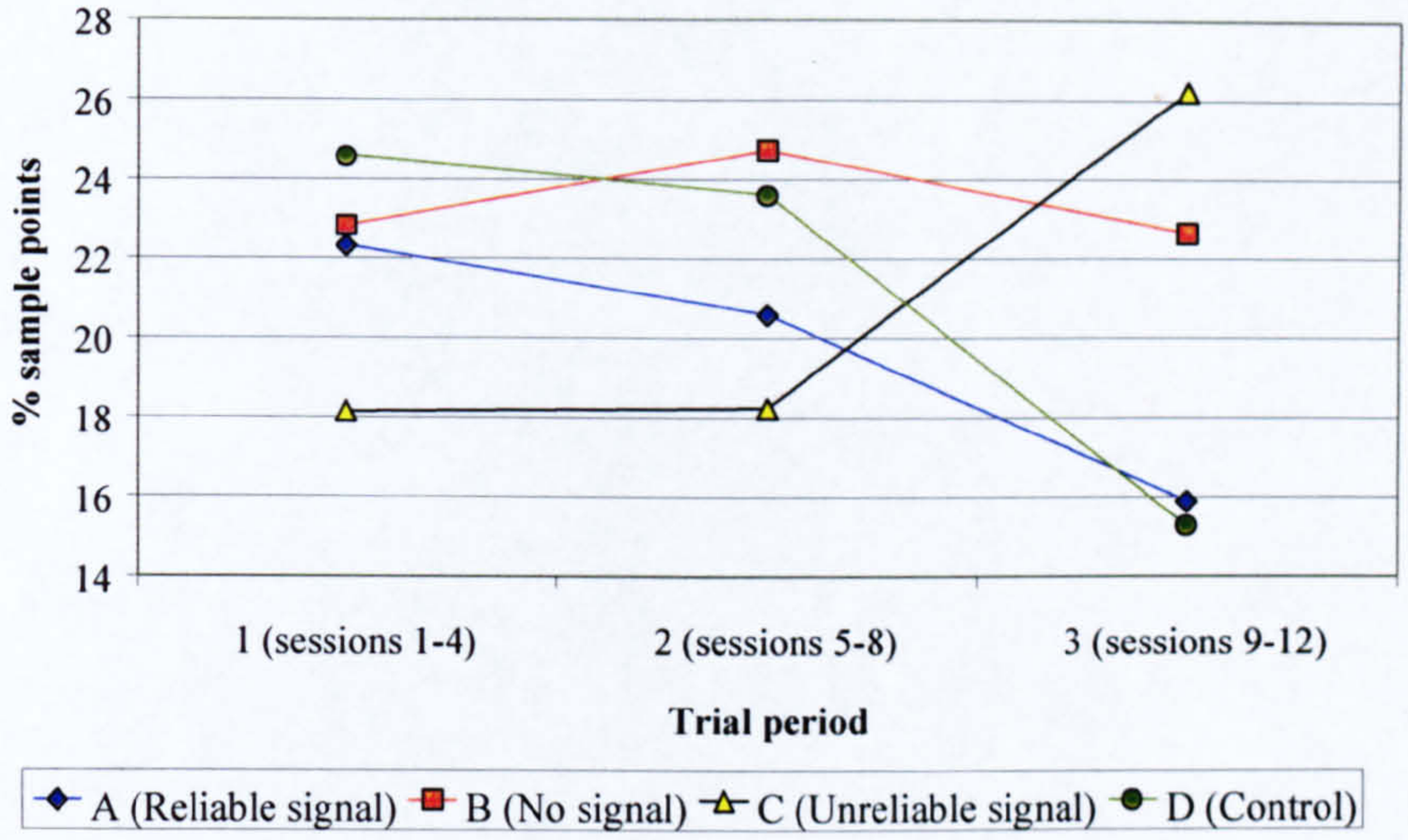
Figure 5.8 Interaction between 'Trial Period' and 'Signalled Predictability' for 'Inactive'



Locomote

There was significant interaction between 'Trial Period' and 'Signalled Predictability' (see Figure 5.9 and Table 5.11). The amount of locomotion decreased over trials for Conditions A and D, with the greatest differences occurring between trials 2 and 3 for both conditions. Although there was no difference in amount of locomotion between Trial Periods 1 and 2 for Condition C, in this condition there was an increase in this behaviour between Trial Periods 1 and 3. There does not appear to be an effect of trial period on amount of locomotion in Condition B. During Trial Period 1, levels of locomotion were lowest for Condition C. During Trial Period 3, levels were lowest for Conditions A and D.

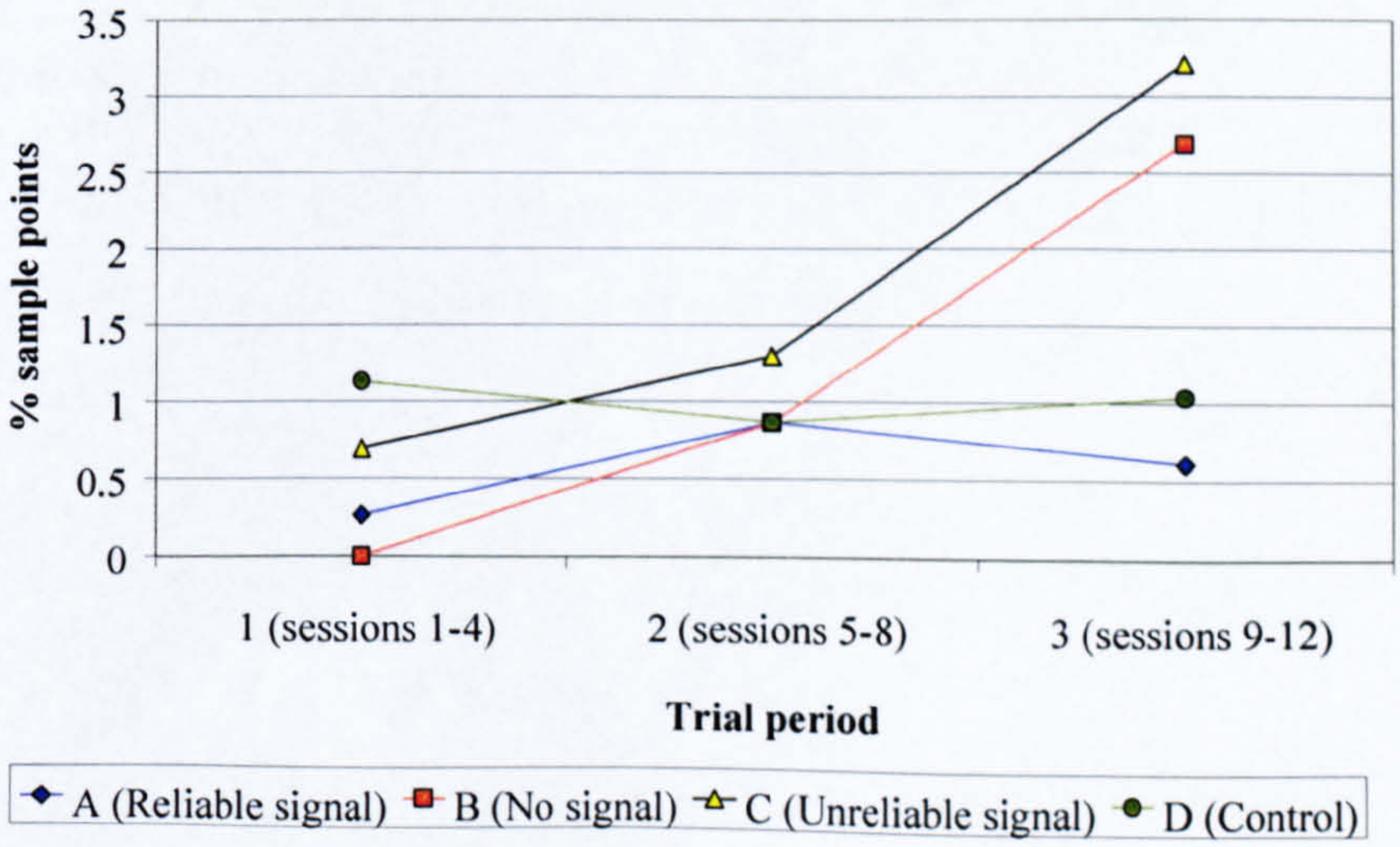
Figure 5.9 Interaction between 'Trial Period' and 'Signalled Predictability' for 'Locomote'



Self-scratch

There was significant interaction between 'Trial Period' and 'Signalled Predictability' for this behaviour (see Figure 5.10 and Table 5.11). There was an increase in the amount of self-scratching over trials for Conditions B and C, but no apparent effect of trial period on scratching for Conditions A and D. For Trial Period 3, levels of scratching were greater in Conditions B and C than in Conditions A and D. This difference was not apparent during Trial Periods 1 and 2.

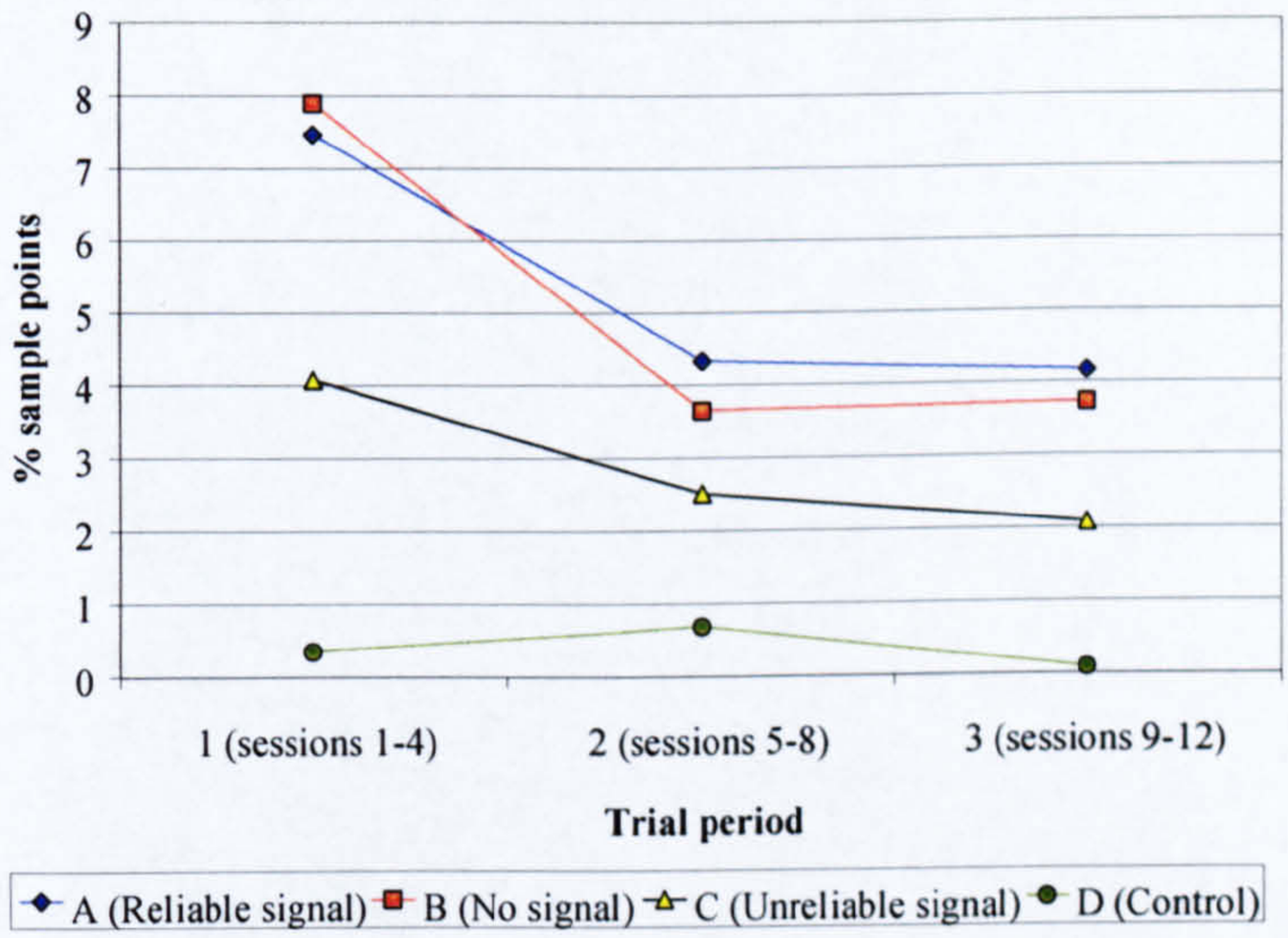
Figure 5.10 Interaction between 'Trial Period' and 'Signalled Predictability' for 'Self-Scratch'



Forage

There was significant interaction between 'Trial Period' and 'Signalled Predictability' (Figure 5.11 and Table 5.11). In Conditions A, B and C, levels of foraging in trial 1 were greater than in trials 2 and 3. This effect was not evident in Condition D. Levels of foraging in all three trial periods were lower in Condition C than Conditions A or B. Levels were lower in Condition D than in any other condition.

Figure 5.11 Interaction between 'Trial Period' and 'Signalled Predictability' for 'Forage'



5.3.4.2 Interactions between 'Signal Period' and 'Signalled Predictability'

There were significant interactions between 'Signal Period' and 'Signalled Predictability' for 'Inactive', 'Locomote', 'Scent mark' and 'Forage' (see Table 5.12). Significant interactions are described.

Table 5.12 Results of ANOVAs for interaction between 'Signal Period' and 'Signalled Predictability' on all behaviours

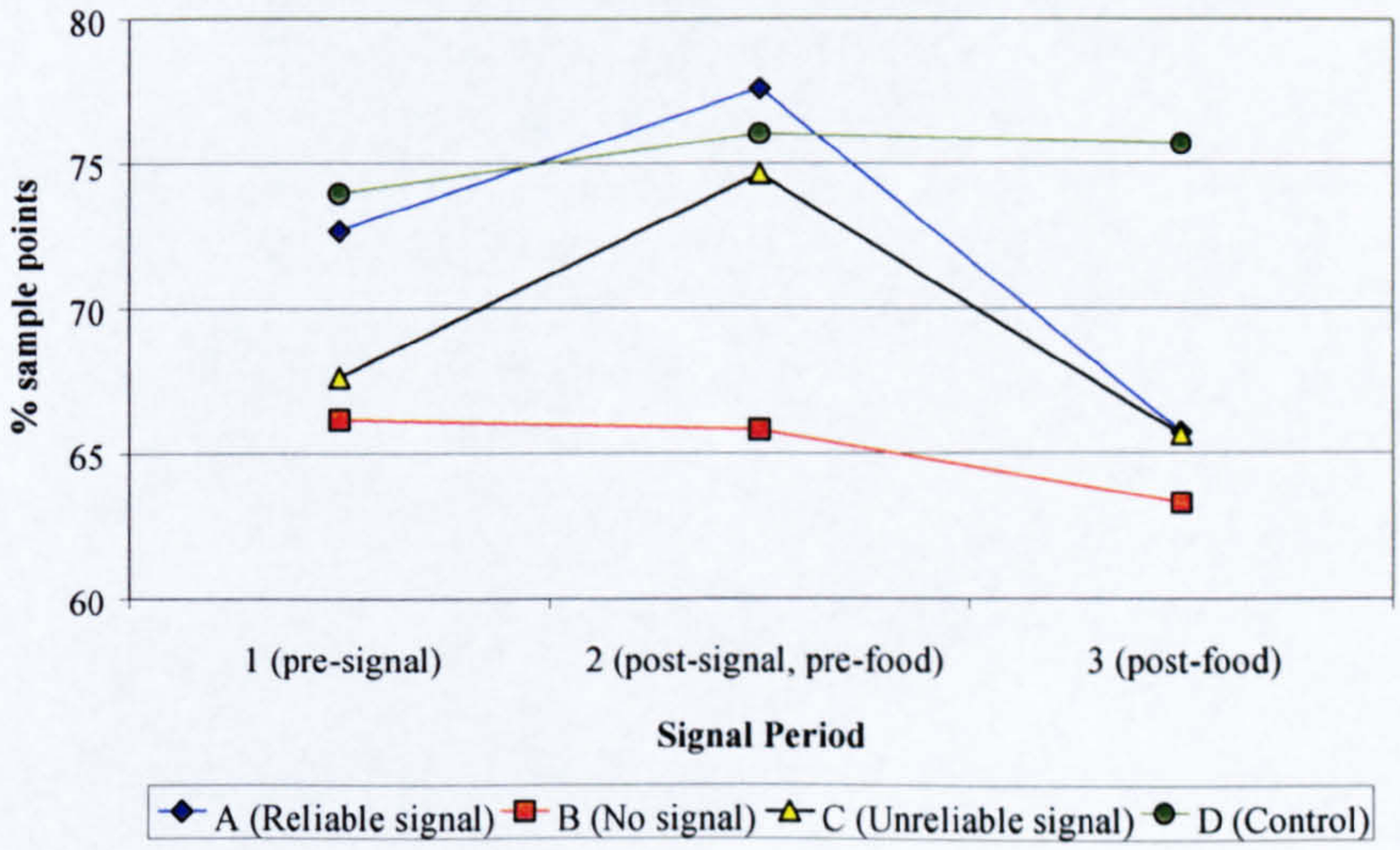
Behaviour	F	p
Inactive	3.59	<0.01**
Locomote	15.64	<0.001***
Self-scratch	1.11	0.06
Scent mark	2.53	<0.05*
Forage	69.95	<0.001***

All d.f. = 6,40

Inactive

There was a significant interaction between 'Signal Period' and 'Signalled Predictability' for this behaviour (Figure 5.12 and Table 5.12). Both Conditions A and C showed a peak in inactivity during Signal Period 2, after the signal but before the food. Condition B animals showed similar, low levels of inactivity throughout all three signal periods. Condition D animals showed similar levels of inactivity across all three trial periods, which were higher than those shown by Condition B animals.

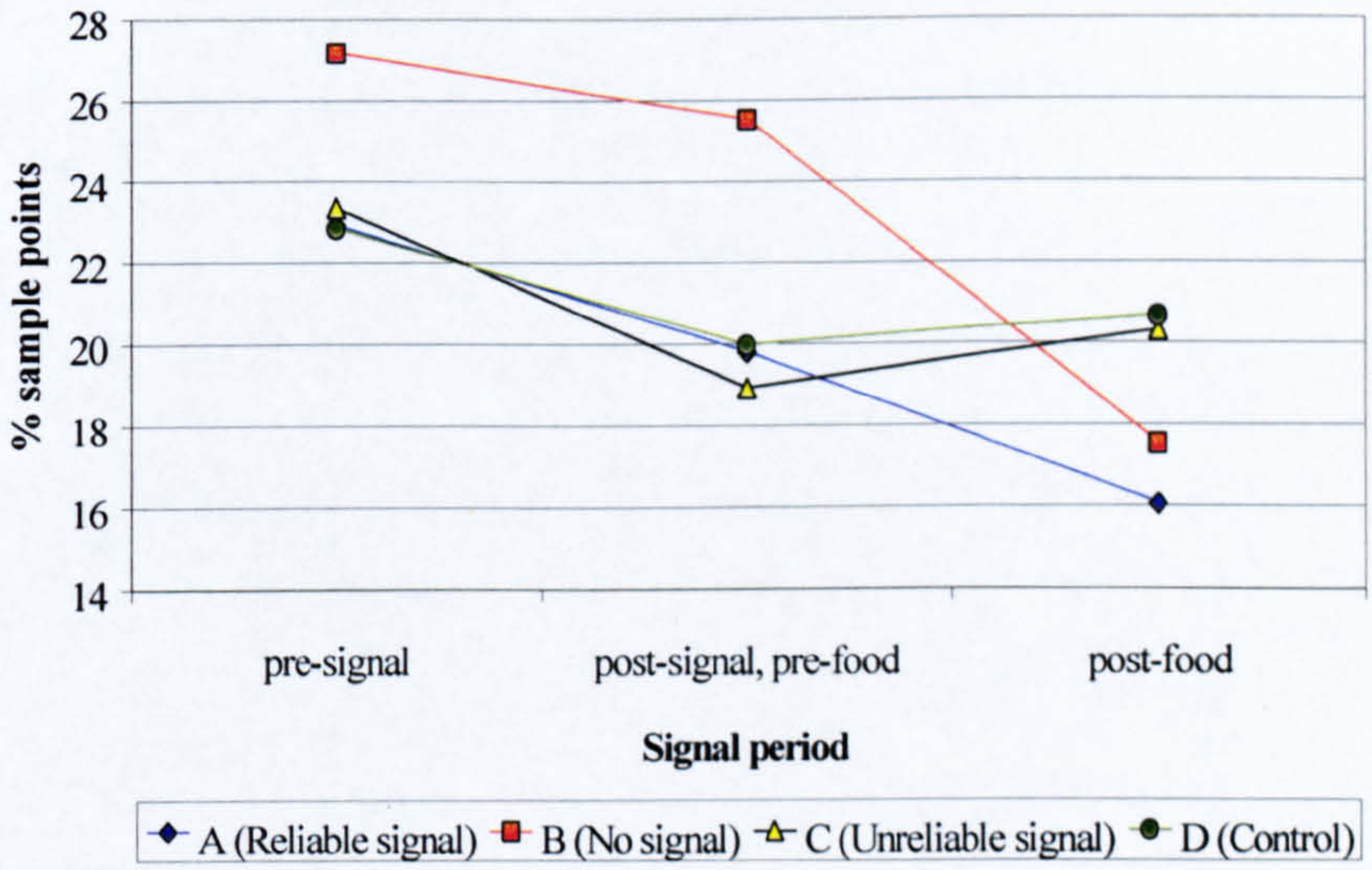
Figure 5.12 Interaction between between 'Signal Period' and 'Signalled Predictability' for 'Inactive'



Locomote

There was a significant interaction between between 'Signal Period' and 'Signalled Predictability' for this behaviour (see Figure 5.13 and Table 5.12). In all conditions, levels of locomotion were higher in the first signal period, *i.e.* before the signal had been given. In Conditions A and B levels of locomotion were lowest in the post-food period (Signal Period 3). However, in Conditions C and D there were no apparent differences in levels of this behaviour between Signal Periods 2 and 3 (*i.e.* between the post-signal, pre-food period and the post-food period). Levels of locomotion were greater in Condition B than the other three conditions, except in Signal Period 3 (post-food), where this difference was absent.

Figure 5.13 Interaction between between 'Signal Period' and 'Signalled Predictability' for 'Locomote'

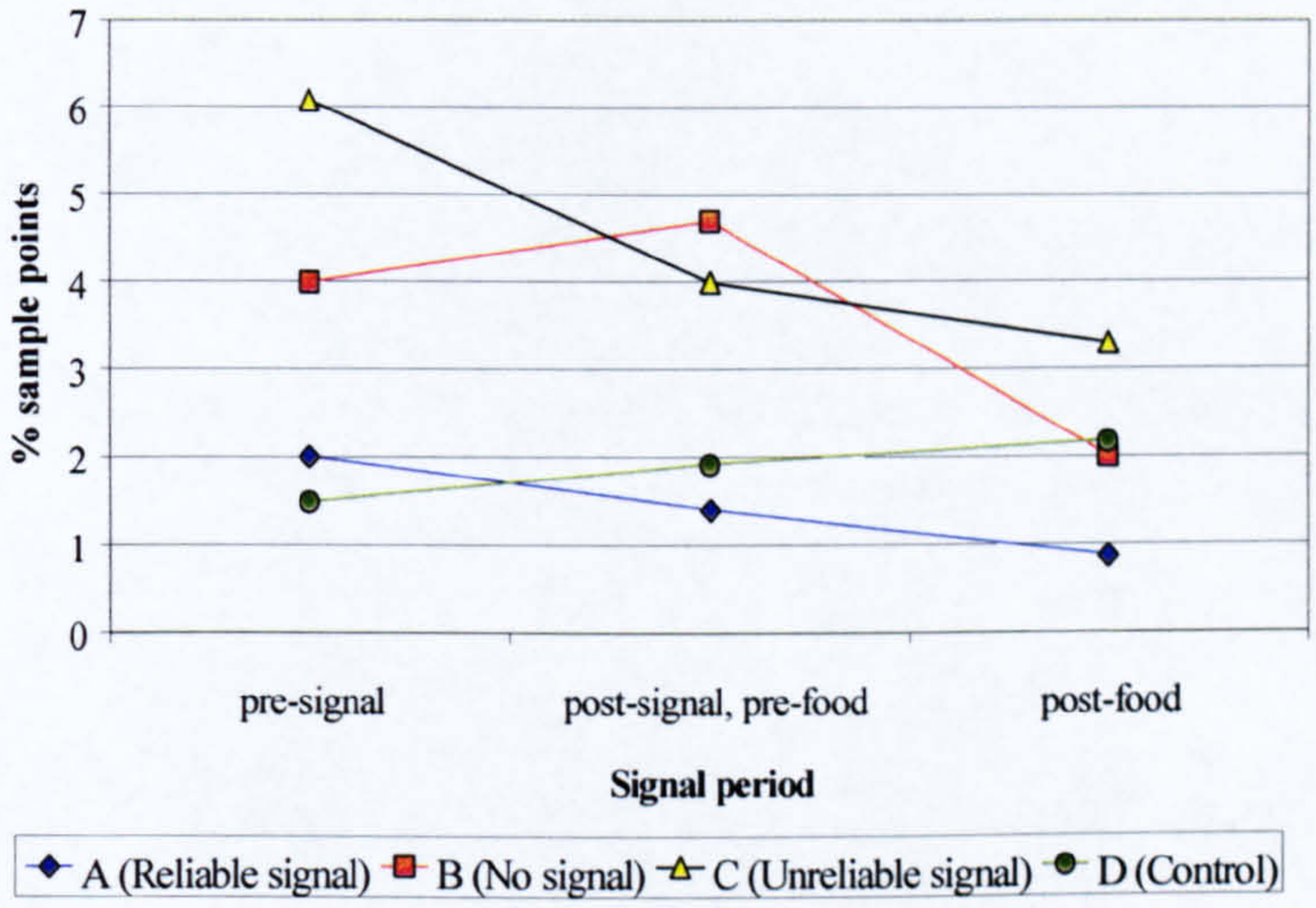


Scent mark

There was a significant interaction between between 'Signal Period' and 'Signalled Predictability' for this behaviour (Figure 5.14 and Table 5.12). Levels of scent marking appeared to decrease in the third signal period (*i.e.* after the food had been given) for Conditions A, B and C. However, this effect was absent in Condition D.

Rates of scent marking were lower in Conditions A and D than in Conditions A and C, except in the post-food period, where these differences were absent.

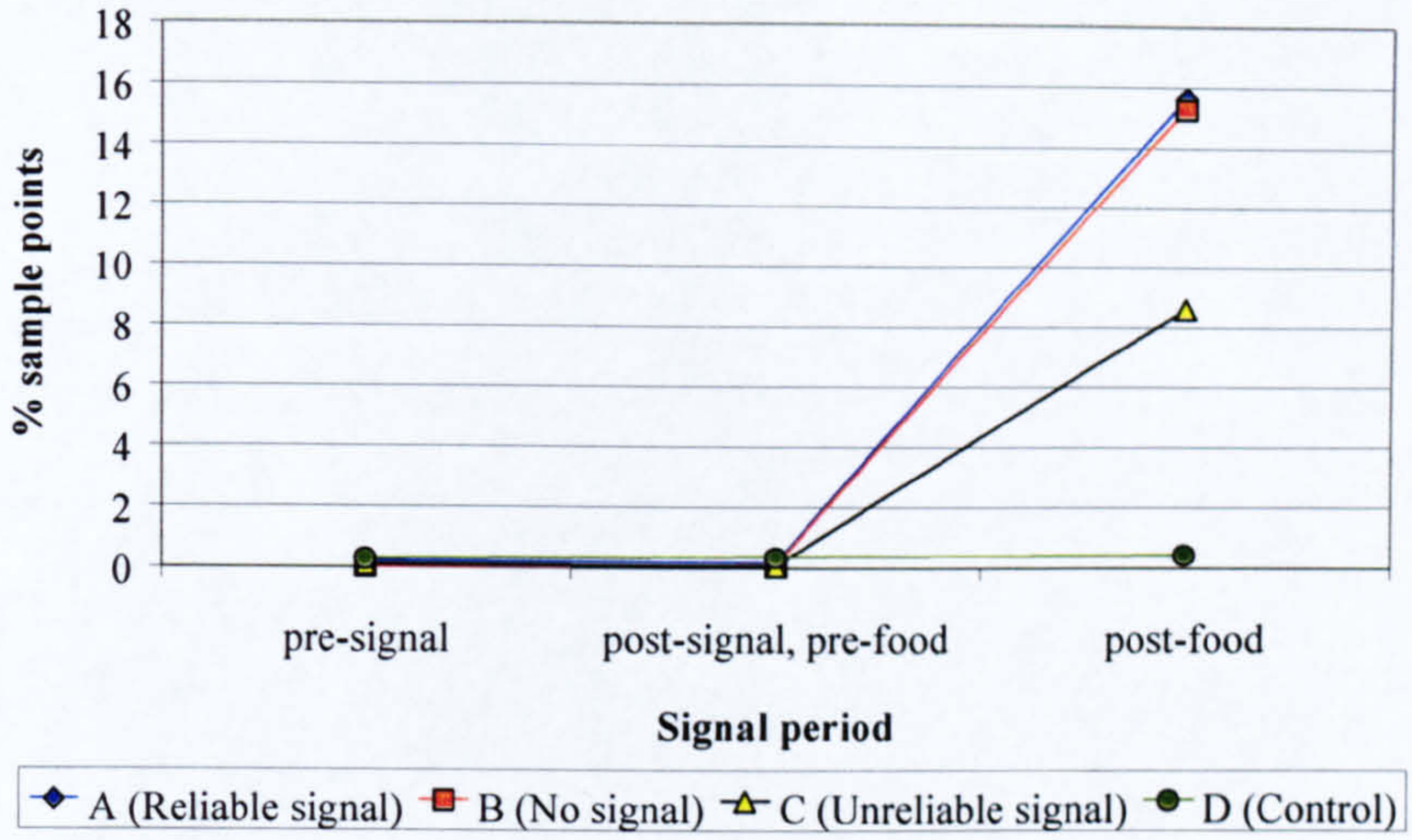
Figure 5.14 Interaction between between 'Signal Period' and 'Signalled Predictability' for 'Scent Mark'



Forage

There was significant interaction between between 'Signal Period' and 'Signalled Predictability' (Figure 5.15 and Table 5.12). In Conditions A, B and C, levels of foraging were greater in Signal Period 3 (*i.e.* after the food had been given) than in Signal Periods 1 and 2 (both before the food had been given). Again, this effect was not apparent in Condition D. There were no differences in amounts of foraging between the four experimental conditions in the first two signal periods (*i.e.* before the food had been given). However, in Signal Period 3 (*i.e.* after the food had been given), levels of foraging were greatest in Conditions A and B, and lowest in Condition D, with Condition C being intermediate between these values.

Figure 5.15 Interaction between between 'Signal Period' and 'Signalled Predictability' for 'Forage'



5.3.4.3 Interactions between 'Trial Period' and 'Signal Period'

There were significant interactions between 'Trial Period' and 'Signal Period' for 'Inactive' and 'Forage' (see Table 5.13). Significant interactions are described.

Table 5.13 Results of ANOVAs for interaction between 'Trial Period' and 'Signal Period' on all behaviours

Behaviour	F	p
Inactive	3.90	<0.01**
Locomote	0.83	0.51
Self-scratch	0.39	0.82
Scent mark	0.49	0.74
Forage	33.48	<0.001***

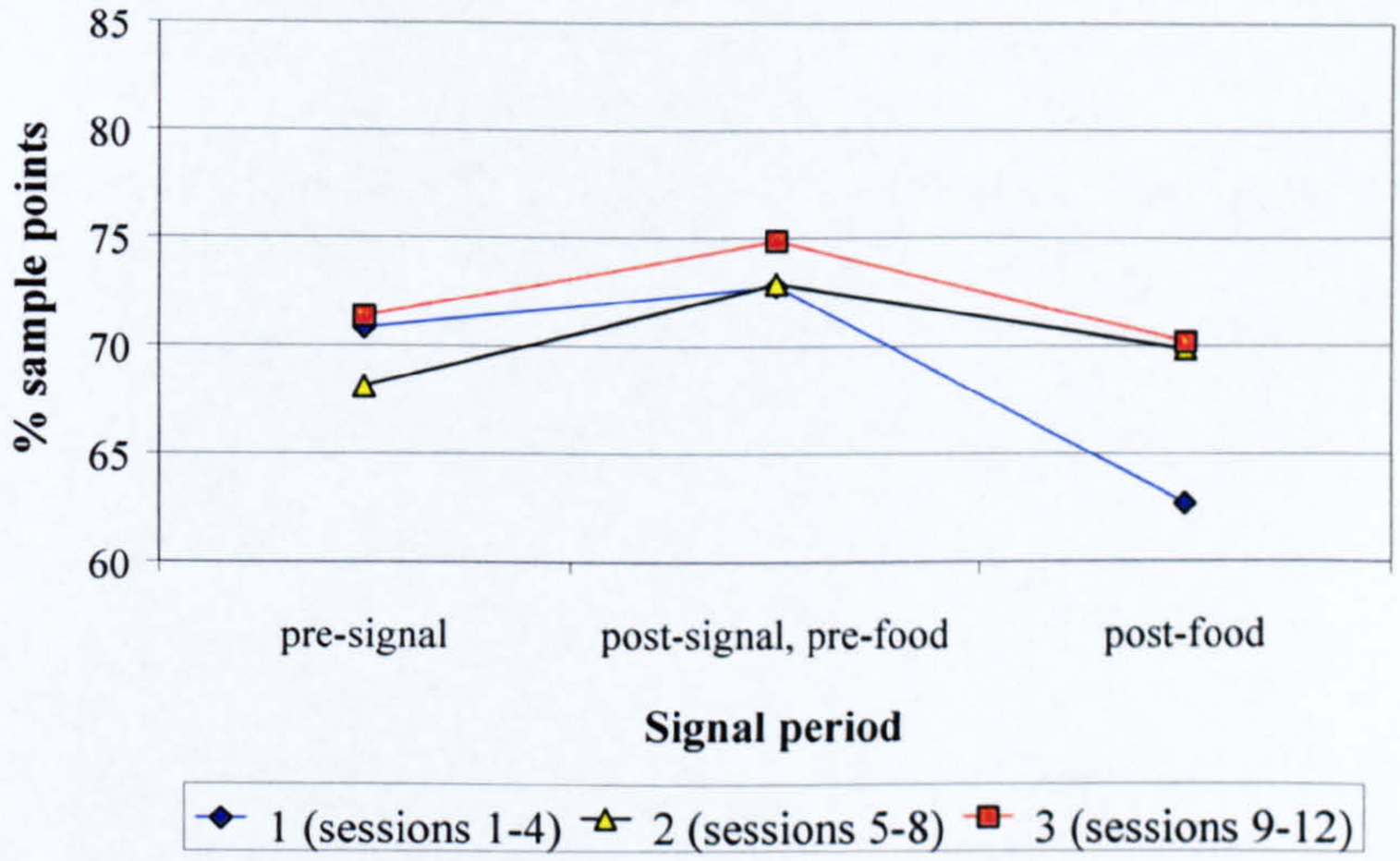
All d.f. = 4,12

Inactive

There was a significant interaction between 'Time Period' and 'Signal Period' for this behaviour (see Figure 5.16 and Table 5.13). In the post-food period, levels of inactivity were lower in Trial Period 1. There was no apparent difference between

the three trial periods at the other two signal periods. In all three trial periods, inactivity was greatest in the post-signal, pre-food period.

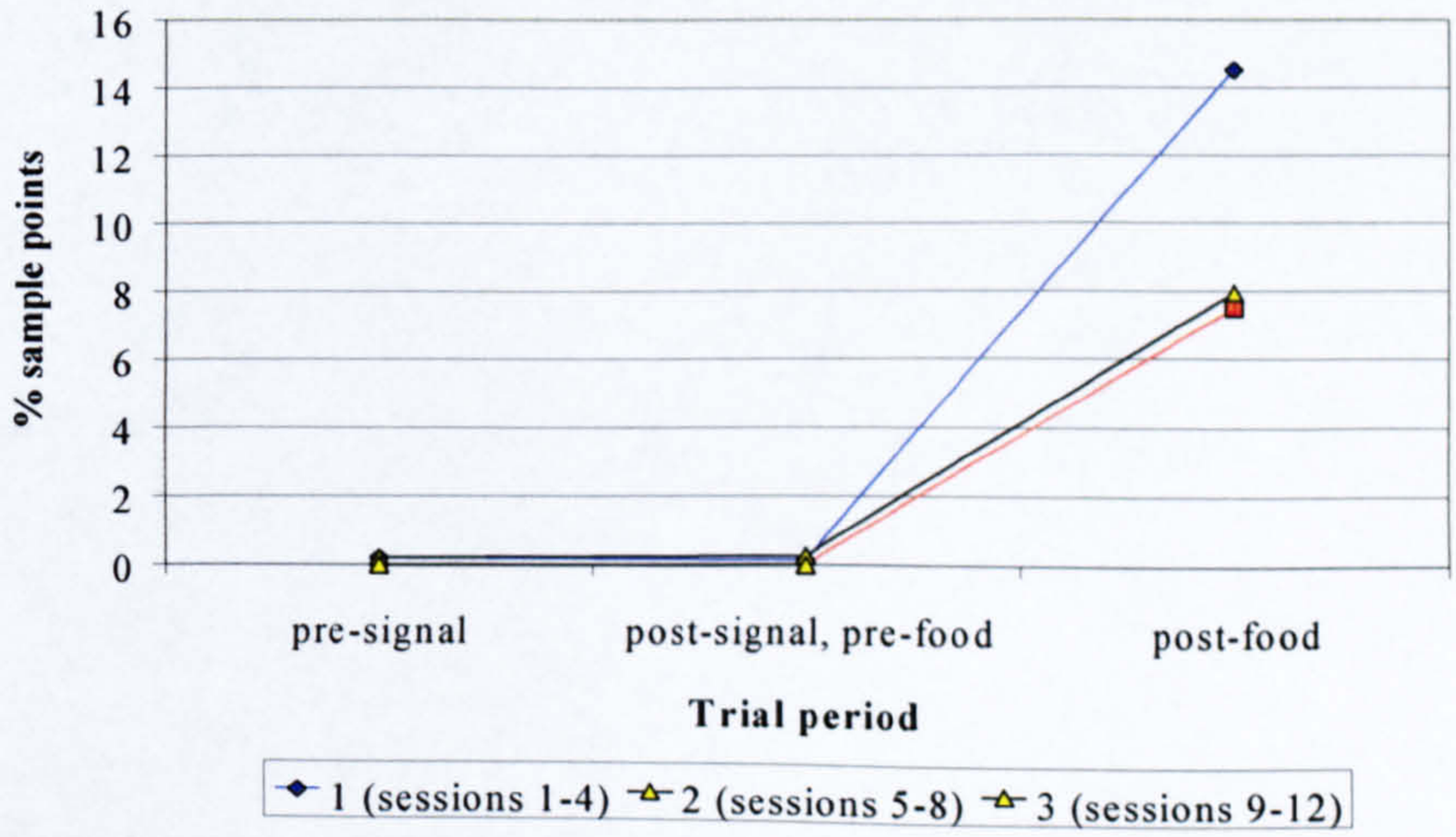
Figure 5.16 Interaction between 'Trial Period' and 'Signal Period' for 'Inactive'



Forage

There was a significant interaction between 'Trial Period' and 'Signal Period' (see Figure 5.17 and Table 5.13). In all three trial periods, there was no difference in amounts of foraging in Signal Periods 1 and 2, before the food had been given. However, in Signal Period 3, after the food had been given, the amount of foraging was greater during Trial Period 1 than during Trial Periods 2 and 3.

Figure 5.17 – Interaction between 'Trial Period' and 'Signal Period' for 'Forage'



5.3.4.4 Three-way interactions between 'Signalled Predictability', 'Trial Period' and 'Signal Period'

The only significant three-way interaction between 'Signalled Predictability', 'Trial Period' and 'Signal Period' was for 'Forage' (see Table 5.14). This interaction is described.

Table 5.14 Results of ANOVAs for three-way interaction between 'Signalled Predictability', 'Time Period' and 'Signal Period' on all behaviours

Behaviour	F	p
Inactive	0.40	0.96
Locomote	0.54	0.89
Self-scratch	1.53	0.13
Scent mark	0.52	0.90
Forage	4.82	<0.001***

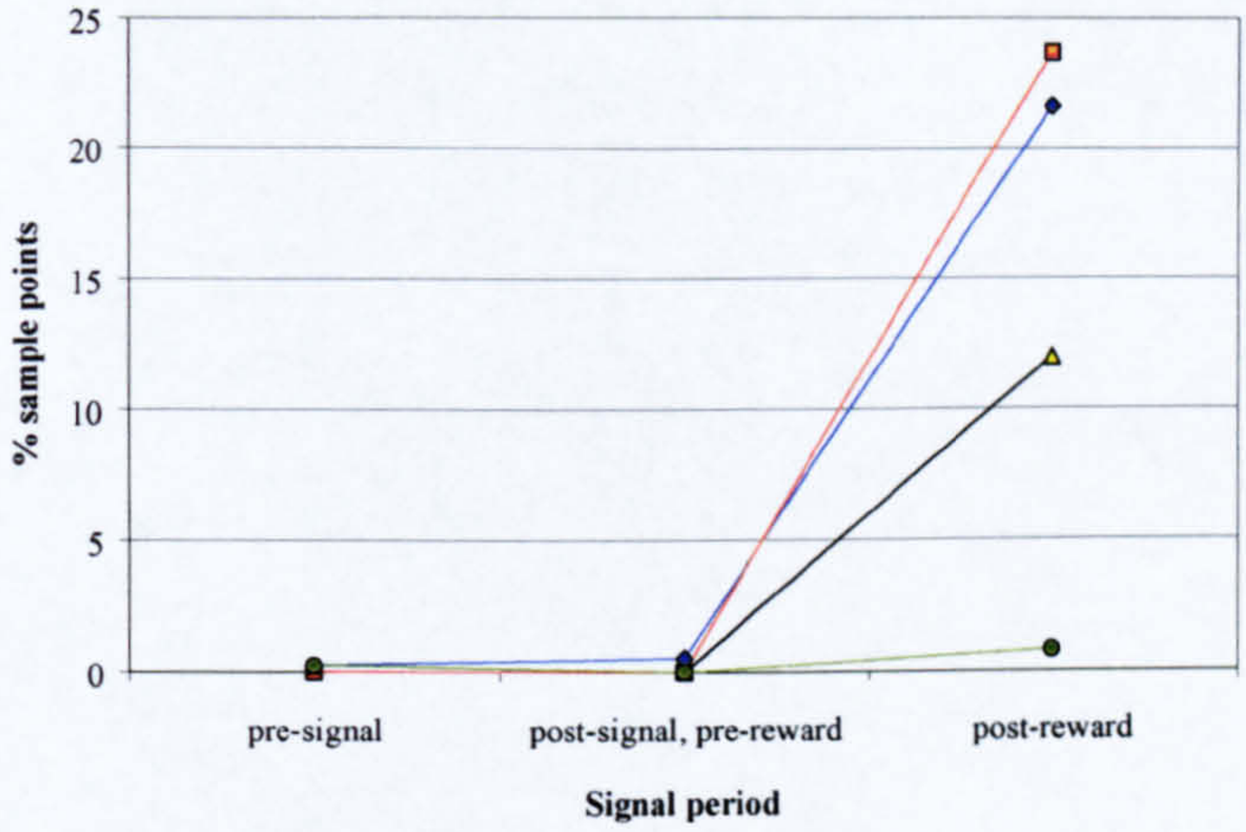
All d.f. = 4,12

Forage

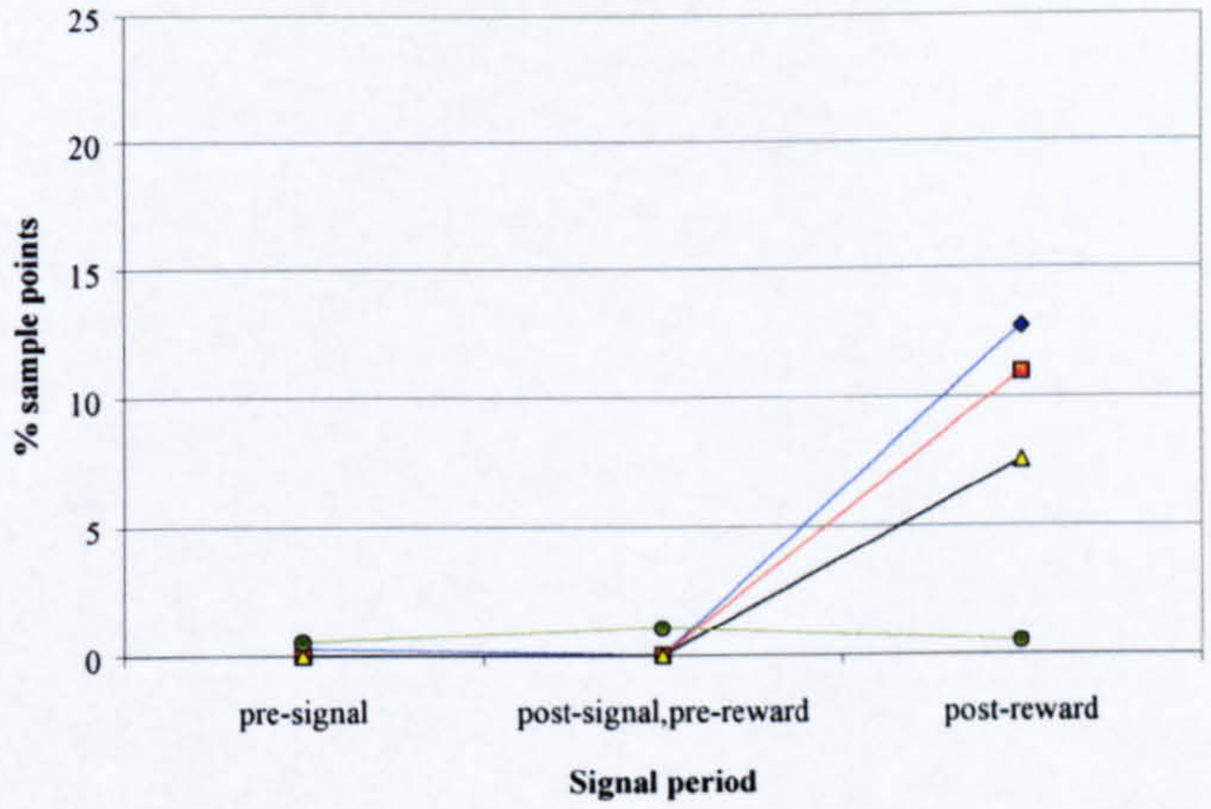
There was a significant three-way interaction between 'Signalled Predictability', 'Time Period' and 'Signal Period' for this behaviour (see Figure 5.18 and Table 5.14). This interaction is shown by three graphs, each representing one trial period (see Fig 5.18). In all three trials, for Conditions A, B and C but not Condition D, levels of foraging in Signal Period 3 were greater than in Signal Periods 1 and 2. Levels of this behaviour were greater for Conditions A, B and C in trial 1 than in trials 2 and 3. In all three trial periods, in Signal Period 3 (post-food), levels of foraging were greatest in Conditions A and B, and lowest in Condition D, with Condition C being intermediate between these values. Neither trial period nor signal period appeared to have an effect on the amount of foraging shown in Condition D (the control condition).

Figure 5.18 Interaction between 'Signal Period' and 'Predictability' for each of the three trial periods for 'Forage'

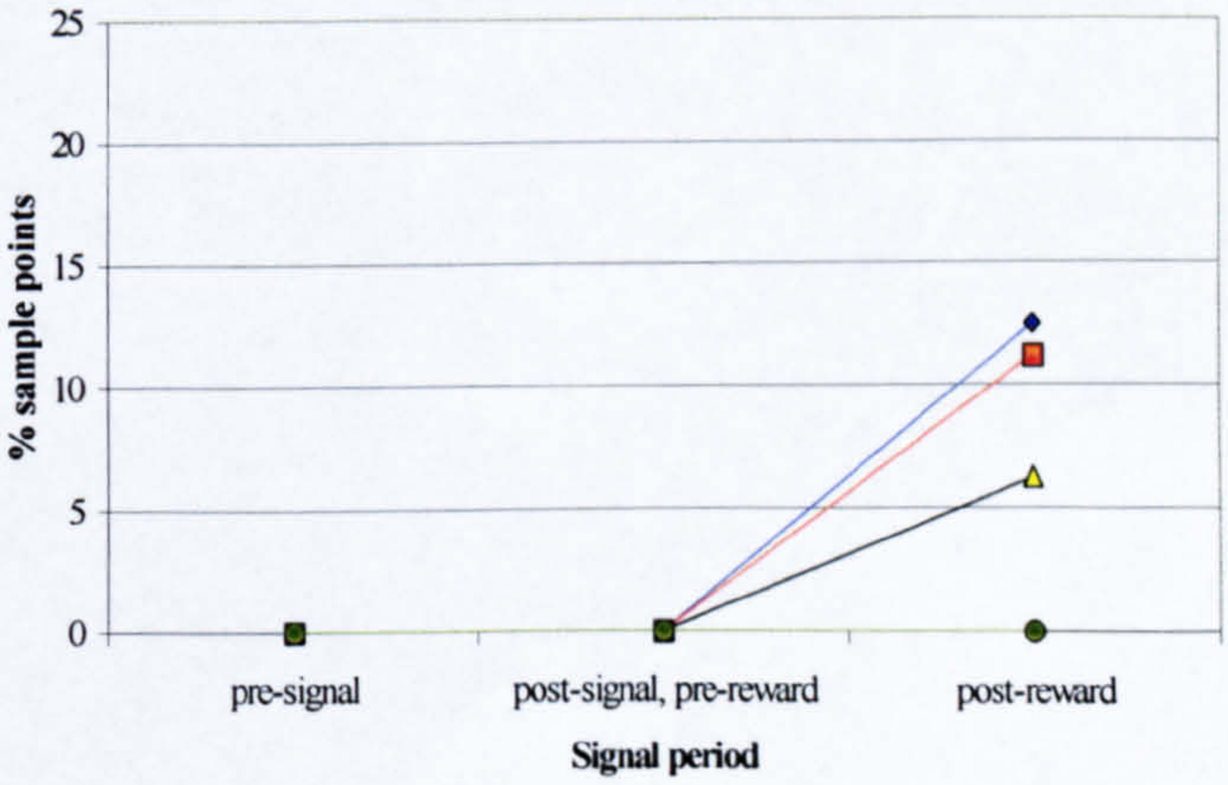
a. Trial Period 1 (sessions 1-4)

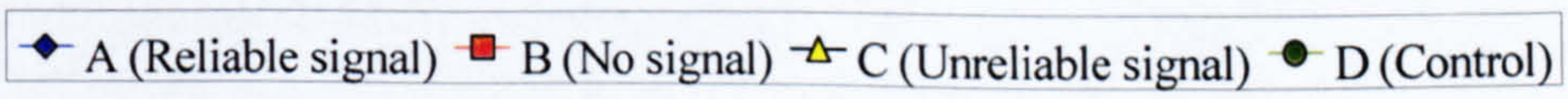


b. Trial Period 2 (sessions 5-8)



c. Trial Period 3 (sessions 9-12)





5.5 DISCUSSION

5.5.1 Changes in behaviour due to habituation to the observer

There was no significant increase or decrease in any of the behaviours studied over the ten-minute period of data collection. There was therefore no behavioural evidence of habituation to the observer over the data collection period. Any changes seen in frequency of behaviour over the individual study periods is therefore likely to be due to experimental manipulations rather than to the presence of the observer.

5.5.2 Effects of food deliveries of varying predictability on behaviour

Inactive

Decreases in inactivity may be associated with stress in the common marmoset (see Chapter 4 for rationale). In this study, the lowest rates of inactivity were shown in Condition B (no signal). Bloomsmith and Lambert (1995) found that predictable feeding was associated with an increase in inactivity; in this study this would appear not to be the case. Predictable feeding here appeared to have no effect on the amount of inactivity shown, whereas unpredictable feeding seemed to be associated with a decrease in inactivity when compared to the control condition. Feeding on an unpredictable temporal schedule without a signal resulted in lower overall rates of inactive behaviour than any other condition, suggesting that this condition was the most stressful.

There was a significant increase in inactivity as trials progressed. When interactions between trial and condition were considered, however, it was seen that only levels in Condition A (reliable signal) and D (control) increased over trials. Levels of activity may be indicators of arousal; in these two conditions, inactivity was lowest in the first few trials, and increased steadily as the study progressed. This may indicate that the experimental procedure caused high levels of arousal, which decreased over trials as the animals became habituated to it. There are strong

similarities between the concept of arousal (*e.g.* Berlyne, 1960) and that of stress (*e.g.* Selye, 1936, 1950); both occur in response to a variety of stimuli and may result in activation of the pituitary-adrenal system (Hennessy & Levine, 1979).

The presence of a human observer in front of the home cage may be stressful for primates. For example, a group of pied tamarins *(Saguinus bicolor bicolor)*, a callitrichid species, on display to zoo visitors displayed more threats, piloerection and approaching than did a group that were not on show (Wormell *et al*, 1996). These results were interpreted as showing that exposure to zoo visitors was stressful to the animals (Wormell *et al*, 1996), although this effect is thought to be influenced by a number of variables such as differences between species and housing conditions (review by Hosey, 2000). Small, arboreal primates such as the common marmoset may be more affected by human presence than larger, terrestrial species, as they may perceive humans as potential predators (Chamove *et al*, 1988; Hosey, 2000). In laboratory studies, the presence of a human observer has been used as an anxiety-eliciting stimulus in a study with common marmosets (Carey *et al*, 1992).

Habituation is said to occur when repeated exposure to a stimulus results in decreased responsiveness to it (Dickinson, 1980; McFarland, 1993). In the present study, repeated exposure to both the experimenter and the experimental situation may have resulted in decreased stress, and lower frequencies of stress-related behaviours exhibited by the monkeys, as trials progressed. No longitudinal studies have so far been carried out to investigate whether habituation to human observers is seen in captive primates (Hosey, 2000). However, the increase in inactivity that occurred in Condition A and D animals suggests that it may have been occurring, at least in these conditions, in the present study.

Levels of inactivity in Condition C (unreliable signal) decreased over trials. This may have been due to arousal, associated with the unpredictable food delivery, increasing as trials progressed. This level of arousal may have been enough to overcome the decrease in arousal, possibly associated with habituation, seen in

Conditions A and D. There was no difference in levels of inactivity over trials in Condition B (no signal). This may have been due to the increase in arousal, associated with the unpredictable food, being cancelled out by the decrease in arousal, due to habituation to the experimental situation. These results suggest that receiving food preceded by a reliable signal was the least stressful for the marmosets, their behaviour changing little from that of control animals.

The most stressful condition appeared to be the no signal condition, although stress levels did not appear to rise over the study period for these animals, as their inactivity levels remained similar throughout the study. The unreliable signal condition was intermediate in terms of inactivity levels and presumably stress between the other two experimental conditions. However, levels of inactivity were reduced over the course of the study, suggesting that this condition became cumulatively more stressful as the study progressed. It is possible that, had the study continued, this trend may have continued in the 'unreliable signal' condition, resulting in less overall inactivity, and higher stress in these animals than was seen for those in the 'no signal' condition.

It is unsurprising that levels of inactivity did not differ between the three signal periods in those animals (Conditions B and D) that did not receive a signal. It is likely that the differences that were seen (levels were higher in the post-signal, pre-food period for both the conditions, A and C, that received a signal) were due to the presence of the signal. Levels of inactivity may have been highest in the post-signal, pre-food signal condition, when a delivery of food was imminent, as animals were simply waiting at the front of the cage for the food to be delivered.

Locomote

There were no overall effects of either condition or trial period on locomotion. However, there was a significant interaction between the two, with an increase in locomotion over trials for Condition C (unreliable signal) compared to a

decrease over trials in Conditions A (reliable signal) and D (control). This is the inverse of the pattern seen for 'inactive'. As increases in locomotion, and decreases in inactivity, are thought to be indicative of increased stress and decreased welfare in this species (see Chapter 4), the conclusions drawn from the interaction between predictability and trial period for locomotion are similar to those for inactive behaviour.

Levels of locomotion were higher in the pre-signal period than in the other two signal periods, although this difference was not as pronounced for Condition C (unreliable signal) or the control condition. The reduction in locomotion in the post-food period for Conditions A (reliable signal) and B (no signal) may have been due to the fact that these animals would always have just received food at this point; this may have lessened anxiety and so reduced locomotion. Alternatively, the fact that food had been given may have meant a significant fall in locomotion because they were occupied in actually eating the food.

Animals in the control condition received neither signal nor food and hence no change in behaviour would be expected between the three signal periods. Those in Condition C (unreliable signal) only received food on 50% of trials; the lack of food on some trials may be the reason that locomotion was not reduced in the third signal period. However, if unpredictable stimuli are stressful, and locomotion is an indicator of anxiety, we would expect levels to be at least as high in Condition C (unreliable signal) as in Condition B (no signal). It is difficult to explain, therefore, why levels of locomotion are almost identical in Condition C to those in the control condition. It appears that more research is necessary to validate the use of locomotion as an indicator of anxiety in marmosets.

Self-scratch

In the present study, the fact that frequency of scratching was significantly higher in Condition C (unreliable signal) than in either the control condition or

Condition A (reliable signal) suggests that unpredictability of food delivery resulted in the highest levels of stress for animals exposed to an unreliable signal. Scratching increased over the course of the study, but the significant interaction between trial and condition indicates that this was only the case for Condition B (no signal) and Condition C (unreliable signal). Levels of anxiety seemed to increase over the study for animals in these two unpredictable food delivery conditions, whereas there was no apparent change for animals in Condition A (reliable signal) or the control condition. Rates of scratching in the reliable signal condition remained similar to those in the control condition throughout the study, suggesting that preceding a temporally unreliable food delivery with a reliable signal makes it less stressful than either not using a signal at all, or using an unreliable one.

There appeared to be no difference in levels of scratching between the four conditions in Trial Periods 1 and 2; differences only become apparent in Trial Period 3. An explanation for this could be that the learned association between the unconditioned stimulus (US; the signal) and the conditioned stimulus (CS; the food) did not become fully established until the third trial period. Anxiety may then have developed only as the relationship between CS and US, and hence predictability of food delivery, were learned. Alternatively, repeated exposure to the experimental procedure may have cumulatively increased anxiety for those animals, in Conditions B and C, that were given food unpredictably. It is possible that increased scratching was only observed when anxiety reached a certain 'threshold' level, and that this level was not attained until the third trial period.

Scent mark

Scent marking was significantly higher in the unreliable signal condition than in the control condition, which suggests, in common with the scratching data, that anxiety was highest in this condition due to the increased unpredictability of the food delivery. Scent-marking was also higher, but not significantly so, in the

condition without a signal and hence no predictive cues as to the timing of the food – if decreased predictability is associated with increased anxiety, an increase in scent-marking would be expected in this condition. The non-significance of this result may be due to the interaction between that was seen between signal period and predictability. When all signal conditions were taken together, scent marking was greater in the pre-signal period than in the post-food period. However, the interaction graph shows that scent marking was higher in the two unreliable signal conditions for both the pre-signal and post-signal, pre-food periods, but not the post-food period. The fact that all three signal periods were taken together to test for effects of predictability of food delivery, and there was no difference between the non-signal condition and the reliable signal condition, may be the reason that the overall difference between this condition and the control was not significant.

It appears that anxiety may have been higher in the two unreliable food delivery conditions, but only before the food was given. Once the food had been given, anxiety was reduced to a similar level to the control condition. However, in the post-food period, scent marking remained higher in the unreliable signal condition than in the other three conditions. This may be due to the fact that food was only given to the animals in this condition on 50% of trials; it is possible that anxiety in the animals that were not fed remained at a higher level than in those animals that had received the expected food. Heightened anxiety may therefore have been a result of the reinforcement schedule of food delivery rather than the reliability of the signal preceding feeding.

Forage

Animals in Conditions A (reliable signal) and B (no signal) received food on all trials, whereas those in Condition C (unreliable signal) received it on only 50% of trials, and those in Condition D (control) did not receive food at all. The differences in amount of foraging between the conditions are therefore likely to be a

result of the availability of food. During the study, the marmosets foraged very little amongst the woodshaving floor covering of the cage, despite the fact that there were generally some leftovers from the previous feed available.

Similarly, the fact that foraging was greatest in the post-food period, as opposed to the pre-signal and post-signal, pre-food period, is due to the fact that this was when the titbits were available. It is less easy to explain, however, why there was a significant reduction in foraging over trials. One possibility for this is that the food given was relatively novel at the start of the study. Primates are fairly conservative in their choice of foods, and exhibit neophobia by avoiding novel foods (Visalberghi, 1994). This effectively reduces the risk of them ingesting poisonous foods. The novelty of the marshmallow given in the study may have made the marmosets initially more cautious about eating this food, and consequently taken them longer to eat it than later in the study, by which time the food had become familiar.

Feeding on an unpredictable temporal schedule preceded by a reliable signal resulted in few differences in behaviour compared to the control condition. This supports the hypothesis that a feeding schedule of intermediate predictability may be most beneficial in terms of animal welfare. Feeding on an unpredictable temporal schedule with no signal preceding food delivery resulted in significantly less inactivity than was seen in the control condition, possibly as a result of arousal due to the unpredictable food. Frequency of scratching increased over trials for this condition, suggesting that stress associated with this feeding schedule increased over time. Feeding preceded by an unreliable signal resulted in increases in locomotion, scratching and scent marking, as the study progressed. All these behaviours are possible indicators of anxiety in common marmosets. The frequencies of scratching and scent marking were significantly higher than in the control condition. Inactivity decreased as trials progressed for control animals.

The combined results of this study suggest that feeding marmosets on an unpredictable temporal schedule may be stressful for them. This may not necessarily be bad for welfare, as some theorists suggest that low levels of stress may actually be beneficial for animals (Wiepkema & Koolhaas, 1993). Preceding feeding with a reliable signal effectively removes this stress, yet preceding it with an unreliable signal increases stress.

Eating is thought to be motivated by both positive and negative affect (Fraser & Duncan, 1998). Negative affect is thought to have evolved to motivate animals in 'need situations' in which a particular action is necessary in order to cope with a threat to survival. Hunger is a negative affective state, which results in motivation to eat. Positive affective states, by contrast, have been favoured by natural selection in 'opportunity situations' (Fraser & Duncan, 1998). The marmosets in this study are unlikely to have been very hungry, as even in the morning before the main feed of the day, there was generally some food left over from the previous day. However, they were offered highly palatable titbits. The failure to receive such desired food does not represent a threat to survival. However, if it is eaten when the opportunity arises it may result in a fitness benefit. It is likely, therefore, that receiving a titbit is reinforcing for the marmosets, and associated with a positive affective state, or 'pleasure'. Therefore, although it has been stated that the 'least stressful' (*e.g.* this chapter, p. 192) condition was that in which the marmosets received food preceded by a reliable signal, this condition might not have been perceived as stressful at all. It may be the case that the experimental procedure in some of the conditions was actually pleasurable to them.

There are unfortunately several shortfalls of this study. For example, it is hard to say whether the schedule used was actually perceived as temporally unpredictable by the animals, as all food was delivered within a two and a half minute 'window' during the eight minutes of data collection. It is therefore

spurious to extrapolate the findings to more temporally unpredictable real-life situations, where food delivery might be varied by a matter of hours. In contrast, if the routine *was* temporally unpredictable to the animals, it is unclear whether similar results would be obtained if food were presented on a temporally predictable schedule with the inclusion and manipulation of the same signals. The next study (Chapter 6) attempts to address this question by incorporating both temporally predictable and unpredictable schedules.

This study would also have benefited from the inclusion of a condition where a previously reliable signal became unreliable, as it would have given an insight into the importance of loss of predictability, which may be more detrimental to welfare than lack of predictability (Mineka & Kihlstrom, 1978). This point is also addressed in the following study (Chapter 6), where an identical protocol is used, but a further four trials without food delivery are included at the end to look at the effects of loss of predictability of feeding. It would also be interesting to look at behavioural changes in response to a change from unpredictable to predictable feeding. It has been suggested that it may take animals longer to physiologically adjust to, or recognise, a predictable routine after being exposed to an unpredictable one than vice versa (Reneerkens *et al*, 2002).

Another major drawback of this study is that it does not specifically address one of the important ideas raised in Chapter 1, that the inclusion of a unique signal might 'buffer' animals against the detrimental effects of disruptions to predictability of feeding. In order to investigate this, a further condition might have been added to the study, in which animals received food on 50% of trials, but food was preceded by a reliable signal; that is, the signal was only given on trials where food was actually delivered. If the signal was found to have beneficial effects in this context, it would highlight the potential benefits of including signals before feeding in real life situations where animals might be affected by delays to feeding (*e.g.* Waitt *et al*, 2001). Feeding would become more predictable in that it would only occur after

the signal. Further research into the potential buffering effects, on the negative consequences of feeding delays, of feeding related signals would be extremely useful.

Finally, it should be noted that in the present study, there is a potential confound associated with one of the conditions. Animals in Condition C received food on 50% of trials, whereas animals in the other two experimental conditions received food on every trial. It is possible that the negative effects on behaviour that were attributed to the unreliable signal, may simply have been due to the different reinforcement schedules used. The following study addresses this variable in particular, in relation to temporal predictability of feeding. However, it would have been useful to have controlled for this in the present study, by including extra conditions with a 50% reinforcement rate and varying degrees of signalled predictability.

5.6 SUMMARY AND CONCLUSIONS

The results of this study suggest that food delivered on an unpredictable temporal schedule and preceded by unreliable signals may result in anxiety and reduced welfare for common marmosets. This is particularly relevant, as it is analogous to a situation often found in real life husbandry systems. Animals may hear sounds they have come to associate with food delivery, such as buckets being rattled, but there may be a delay or food may be given to their neighbours instead. Food delivered on an unpredictable temporal schedule but not preceded by a signal resulted in some activation of proposed stress indices, but to a lesser extent than was seen in the 'unreliable signal' condition. The eradication of sounds associated with food preparation and delivery may therefore represent an improvement in welfare where delays are likely or where several groups of animals within auditory range are to be fed. However, this is not a practical solution in most husbandry systems. Food delivered on the same schedule but reliably preceded by a unique signal results in

little change in behaviour compared to animals left undisturbed. The reliable sounding of a unique signal for each group of animals, a certain time period before food is given, may be beneficial in extinguishing previously learned associations between food-associated sounds and food delivery. This may result in a reduction of stress and an improvement of welfare for animals so housed.

Chapter 6

Effects of temporal predictability of feeding on the behaviour and welfare of common marmosets

6.1 INTRODUCTION

The study described in the previous chapter explored the effects of manipulating the presence and reliability of a feeding related signal. The present study, in common with the previous one, investigated the effects of predictability of feeding on behaviour. However, in contrast to the previous study, signals were not used. Here the predictability of feeding was manipulated in two concurrent ways. Firstly, the temporal predictability of feeding was varied by delivering the food at a fixed or variable time on each trial. This variable was not manipulated in the previous study, being unpredictable in all conditions. It is likely, however, based on the findings of other studies (Bloomsmith & Lambert, 1995; Carlstead, 1998; Krishnamurthy, 1994; Waitt & Buchanan-Smith, 2001; Wasserman & Cruikshank, 1993) that the temporal predictability of food delivery will have significant effects on the behaviour and, by analogy, the welfare, of captive animals. Secondly, the non-contingent reinforcement schedule was also manipulated, by giving food on 50% or 100% of trials. This was a factor in the previous study, as in the 'unreliable signal' condition, food was delivered on 50% of trials. However, the effects of different reinforcement frequencies were not specifically addressed.

It has been stated that signals *per se* were not used or manipulated in the present study. However, the trials were not completely free of feeding-related cues, as my presence in front of the cage was such a signal. This was unavoidable in terms of the experimental set-up, yet might be considered to be beneficial to the study in that my presence might be regarded as analogous to non-specific feeding-related cues that are generally inevitable in real-life husbandry situations. The experimental trial period was comparable to a 'window' around feeding time,

during which general non-specific feeding related cues might be perceived by the animals. These cues might, for example, be related to other animals being fed, or food being prepared for delivery later. Conditions with food delivery on 50% of trials represented a situation in which feeding occurred on only a 'proportion' of relevant occasions – so feeding-related cues (experimentally, my presence) might be present when no food was available.

The most predictable food delivery occurred in Condition A, when food was given on 100% of trials, each pair of animals receiving it a fixed time on each trial. In Condition B, food was given at a variable time, and on 100% of trials, representing a moderately predictable schedule. Animals in Condition C received food at a fixed time on 50% of trials, again thought to be a moderately predictable condition. In Condition D, the least predictable condition, food was given on a variable time schedule on 50% of trials. Condition E was included as a Control; here, no food was given.

It has been hypothesised (Novak & Drewson, 1989; Wiepkema & Koolhaas, 1993) that an intermediate level of predictability of environmental events, such as feeding, should be most beneficial in terms of welfare. This hypothesis was supported in the previous study, where food delivered on a temporally unpredictable schedule preceded by a reliable signal was associated with the lowest rates of stress-related behaviours. Based on this hypothesis, it was expected that the lowest rates of stress-related behaviours would be seen in the two conditions incorporating moderately predictable schedules. The highly predictable schedule was expected to be associated with increased arousal (Mistleberger, 1994) and stress-related behaviours. Likewise, the highly unpredictable schedule was expected to be associated with an increase in rates of behaviour associated with increased stress, as was seen in the previous study.

In contrast to the previous study, an extra four sessions (comprising one trial period) when no food was given to animals in any condition, were added to the end

of the study. This was in order to assess the impact on behaviour of animals not receiving food in situations where we might expect them to anticipate feeding. It was thought that animals accustomed to being fed on schedules of varying predictability might react differently in the absence of expected food.

The nature of husbandry routines is such that some degree of temporal predictability of feeding is inevitable. The continuum between temporal predictability and unpredictability might be conceived as a continuum ranging between a very short temporal 'window' within which food delivery occurs, and an infinitely large window, which would represent a truly temporally unpredictable routine.

Obviously, when dealing with animals that are fed at least once daily, an infinitely large 'window' is out of the question. Also, because keepers and technicians generally have their own routines to follow, as well as normal working hours outside which feeding is impossible, even the most temporally unpredictable feeding routine will usually have a window of far less than 24 hours. Comparisons between temporal 'predictability' and 'unpredictability' are therefore relative to each other rather than absolute. The final four experimental sessions, when food was not delivered, represented delays to food delivery outside the normal temporal window. For example, if an 'unpredictable' routine involves feeding at a random time between 0900 and 1300h, it might be considered disrupted if, for whatever reason, food was not delivered until 1500h.

It was hypothesised that the greatest increase in stress-related behaviours in response to the lack of food in the final trial period would occur for animals in Conditions A and B. This was because they received food on 100% of the initial trials. It was expected that Condition C and D animals, which had already been subject to trials in which no food was delivered, would be relatively unaffected by the change in reinforcement schedule.

6.2 METHOD

6.2.1 Study animals

Study animals were 60 common marmosets, housed at the MRC Human Reproductive Sciences Unit, Edinburgh. Details of housing and husbandry routines are given in Chapter 3. Twenty-four of the animals had been used in the study detailed in the previous chapter, which had been carried out around nine months earlier. These individuals were evenly assigned to each experimental condition in the present study.

A between-subjects design was used, with six pairs of animals in each of five conditions. Each experimental group consisted of two mixed-sex pairs, two male pairs and two female pairs, as was the case in the study described in Chapter 5. Animals housed in upper-tier and lower-tier cages were split equally throughout the five conditions. The mean age of study animals was 857.90 days (±SE 73.09). Study animals were matched across condition for age; see Table 6.1 for mean ages of animals in each condition. The ages of the animals in each condition were not significantly different from each other (between subjects ANOVA, $F_{4,55}=1.11$; $p=0.36$).

Table 6.1 Mean ages of animals (± Standard Error, S. E.) in each experimental condition at the start of the study (01/06/01)

Condition	Mean age (days)	S. E. of age (days)
A	966.75	197.64
B	814.58	118.20
C	579.00	88.24
D	921.00	211.58
E	1008.167	167.27

6.2.2 Experimental design

The study was conducted in two consecutive stages. Food was provided only in the initial stage, whereas the second stage consisted only of behavioural

observations with no food delivery. The first stage consisted of twelve trials for each pair. There were four trials per pair in the second stage.

Procedure for Stage 1 of the study

Predictability of food delivery was effected by concurrent manipulation of two factors. The temporal predictability (*i.e.* the time schedule) was one factor that was manipulated; the second factor was the proportion of trials in which food was delivered (*i.e.* the non-contingent reinforcement schedule, referred to hereafter as the reinforcement schedule). In common with the study described in Chapter 5, the food used was a small piece of marshmallow dropped into an empty camera film case attached to the front of the cage. Two pieces of food were provided on each occasion, in an attempt to ensure that both animals received one where appropriate. The procedure of delivering the food took less than five seconds on each trial, and anticipatory signals were kept to a minimum. For example, the food was held in the hand so that there would be no need to rummage in a pocket to find it, which might have become a reliable signal that food delivery was imminent.

Animals in Condition A received food on a temporally predictable (fixed time) schedule – food was given after the same interval on each trial. Each pair was assigned one of the following times: 3 minutes 30 seconds, 4 minutes, 4 minutes 30 seconds, 5 minutes, 5 minutes 30 seconds or 6 minutes. Food was delivered to the relevant pair this amount of time from the start of the observation period, when I stood (for upper-tier cages) or sat (for lower-tier cages) directly in front of the relevant cage. Each pair was therefore given a piece of desired food at a temporally predictable time, but the time was different for each pair. The mean time at which food was delivered was therefore 4 minutes 45 seconds. Food was given on 100% of trials (*i.e.* there was a reinforcement ratio of 1). Overall, this was the most predictable of the four experimental conditions, being both temporally predictable and with a 100% certainty of food delivery on every trial.

In Condition B, animals were again given food on every trial. However, here food was delivered on a temporally unpredictable (variable time) schedule, with food delivered after a variable interval on each trial. The same six timings were used as in Condition A, but each pair received food at each of these times twice during the study period. Again, the mean time of food delivery was 4 minutes 45 seconds after the start of the observation period. The order of the timings of food delivery was randomly assigned. This condition was identical to Condition B in the study described in Chapter 5. Predictability in this condition was intermediate in relation to the other conditions, as food delivery was temporally unpredictable but there was a reinforcement frequency of 1.

Condition C was identical to Condition A, except that food was provided on only 50% of occasions; that is, on six out of twelve trials. The reinforcement frequency was therefore 0.5. Trials on which food was delivered were randomly assigned for each pair. On the trials that food was given, it was delivered on a temporally predictable schedule, each pair being assigned one of the six timings above. Again, overall predictability in this condition was intermediate, as a fixed time schedule was used but there was no means by which the animal could predict whether or not food would actually be provided on a given trial.

Similarly, Condition D was identical to Condition B, except that food was provided on only 50% of occasions. This was the least predictable of the four experimental conditions, as it was a variable time schedule with a concurrent reinforcement schedule of 0.5. Condition E was the Control condition. Here animals were observed in the same way as in each of the other four conditions, but never given any food. See Table 6.2 for a summary of the five conditions used in the study.

Table 6.2 Experimental conditions used in the study[1]

Condition	Experimental manipulation *Temporal predictability, reinforcement schedule*	Overall predictability
A	*Predictable, 100%* Animals were given food at the same time on every trial (Fixed time schedule, reinforcement frequency 1)	High
B	*Unpredictable, 100%* Animals were given food at a random time on every trial (Variable time schedule, reinforcement frequency 1)	Moderate
C	*Predictable, 50%* Animals received food on 50% of trials, but at the same time on each occasion (Fixed time schedule, reinforcement frequency 0.5)	Moderate
D	*Unpredictable, 50%* Animals received food on 50% of trials, at a random time on each occasion (Variable time schedule, reinforcement frequency 0.5)	Low
E	*Control* Animals did not receive food on any trial	X

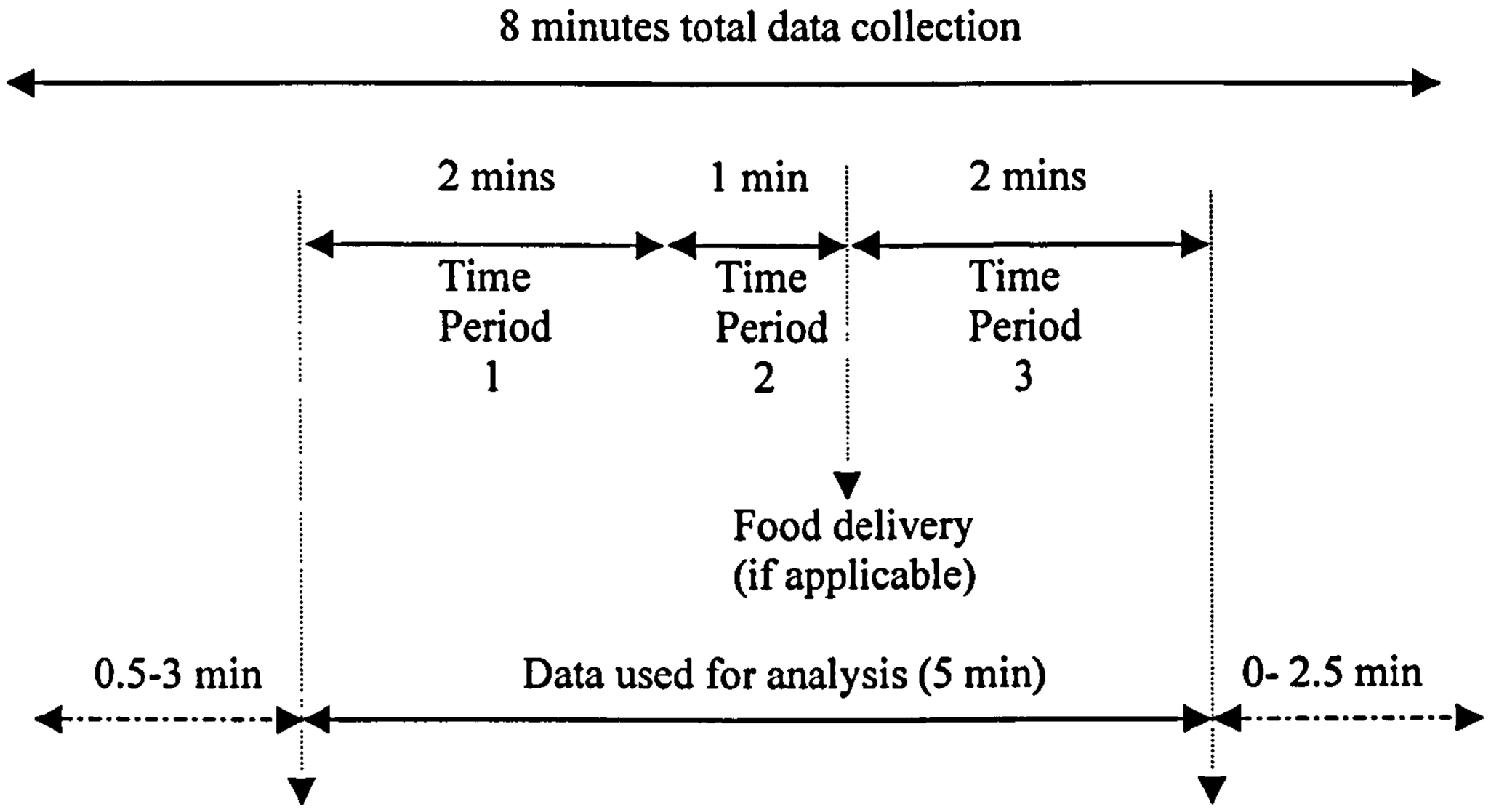
[1] The above experimental manipulations (Conditions A-D) were only used in trials 1-12. In trials 13-16, no food was given to animals in any condition

Procedure for Stage 2 of the study

In Stage 2 of the study, a further four trials were conducted for each pair. However, in these final trials, food was not provided in any of the five conditions. As in the previous study (Chapter 5), each data collection period lasted for eight minutes, which ensured that each pair was exposed to the observer for the same amount of time. In order to make the results of this study directly comparable with those from the previous one, data from three periods in the observation were used for analysis. These were the 2-minute period from three minutes to one minute before food delivery (referred to in the analysis as Time Period 1), the one-minute

period directly before the food was given (Time Period 2) and the two-minute period directly after food delivery (Time Period 3) (see Figure 6.1). The Control group were randomly assigned the same timings for food delivery as Conditions B and D, and data from appropriate periods relative to these used for the analysis, although food was never actually given in this condition.

Figure 6.1 - Time periods used in the study



It was not possible to include data from the previous study in the same analysis as the data from this one. This was because exploratory analyses, using between-subjects ANOVAs, found significant differences between the two Control groups for two behaviours. There was significantly more inactivity ($F_{1,10}$=18.70; $p<0.01$) and less locomotion ($F_{1,10}$=24.55; $p<0.001$) in this study compared with the previous one. It is unclear why these differences should be found, but they could be due to general habituation to me as I had been working at the laboratory for longer, carrying out tasks such as feeding the animals, by the time the second study was carried out. The differences between the data from the two studies mean that they are not directly comparable. However, although the data themselves cannot be compared between studies, the results of these exploratory analyses do not affect the

validity of within-study comparisons. It is also valid to compare general trends found within the two studies.

Table 6.3 Variables in the study, and number of levels within each

Variable	**Within / between subjects**	**No. of levels**	**Levels**	
Temporal predictability of food delivery	Between-subjects	5	Condition A: Predictable 100% Condition B: Unpredictable 100% Condition C: Predictable 50% Condition D: Unpredictable 50% Condition E: Control	
Trial period	Within-subjects	4	Trial period 1: Sessions 1-4 Trial period 2: Sessions 5-8 Trial period 3: Sessions 9-12	Stage 1 (food delivered)
			Trial period 4 - Sessions 13-16	Stage 2 (no food delivered)
Time period in relation to food delivery	Within-subjects	3	Time period 1: 3 min pre-food → 1 min pre-food Time period 2: 1 min pre-food → food Time period 3: Food → 2 min post-food	

It was thought that behaviour might change as the study progressed, as the learned association between time and food delivery might take some time to become established. For this reason, data were split into four chronological periods for the analysis. The first four trials were categorised as Trial Period 1, while the second and third four trial blocks were categorised as Trial Periods 2 and 3 respectively. Trial Period 4 was composed of the last four trials; these were the trials for the second stage of the study, where no food was given in any condition. See Table 6.3 for a summary of the variables under investigation.

6.2.3 Data collection

Data were collected on a palm-top computer, running THE OBSERVER 3.0 programme (Noldus, 1993). Instantaneous scan sampling was used with a 15-second interval between scans. As in the previous study, the behaviours recorded included 'inactive', 'locomote', 'self-scratch', 'scent mark', 'vocalise', 'forage' and 'other' (see Chapter 3, page 89 for behavioural definitions). These categories were mutually exclusive. Vocalisation data were not thought to be relevant to welfare assessment (see Chapter 4 for rationale), and so are not presented in this chapter; instead, they are given in Appendix 2, along with the two sub-categories of inactive behaviour, 'inactive (watching observer)' and 'inactive (not watching observer)'. See Chapter 3 for behavioural definitions.

Sixteen trials were carried out for each pair. The interval between trials was between two and four days in each case. Trials were evenly balanced between morning (1100h – 1230h) and afternoon (1330h – 1500h) sessions. A total of 3840 minutes of data were collected, of which 2400 was used in the analysis. Data collection took place over a ten-week period, between June and August 2001.

6.2.4 Statistical analysis

As in Chapter 5, a single mean was calculated from each pair of animals to be used in the analysis. This was because the behaviour of each individual was likely to have been influenced by its cage mate, and so the data from each individual could not be treated as being independent. Data were normally distributed, so parametric tests were used throughout the analysis.

A three-factor mixed analysis of variance was used to look at changes in behaviour associated with the study variables (see Table 6.3 for a summary of these). Significance was set at alpha <0.05. Percentages of total sample points spent in each behaviour were used, as the time periods under consideration were of different lengths. Post-hoc Tukey tests were used where the between-subjects factor

of predictability of food delivery was significant, in order to compare each condition with the others. Where the within-subjects factors of trial period and time period were significant, post-hoc *t*-tests were used with the appropriate Bonferroni correction to control for possible Type I errors.

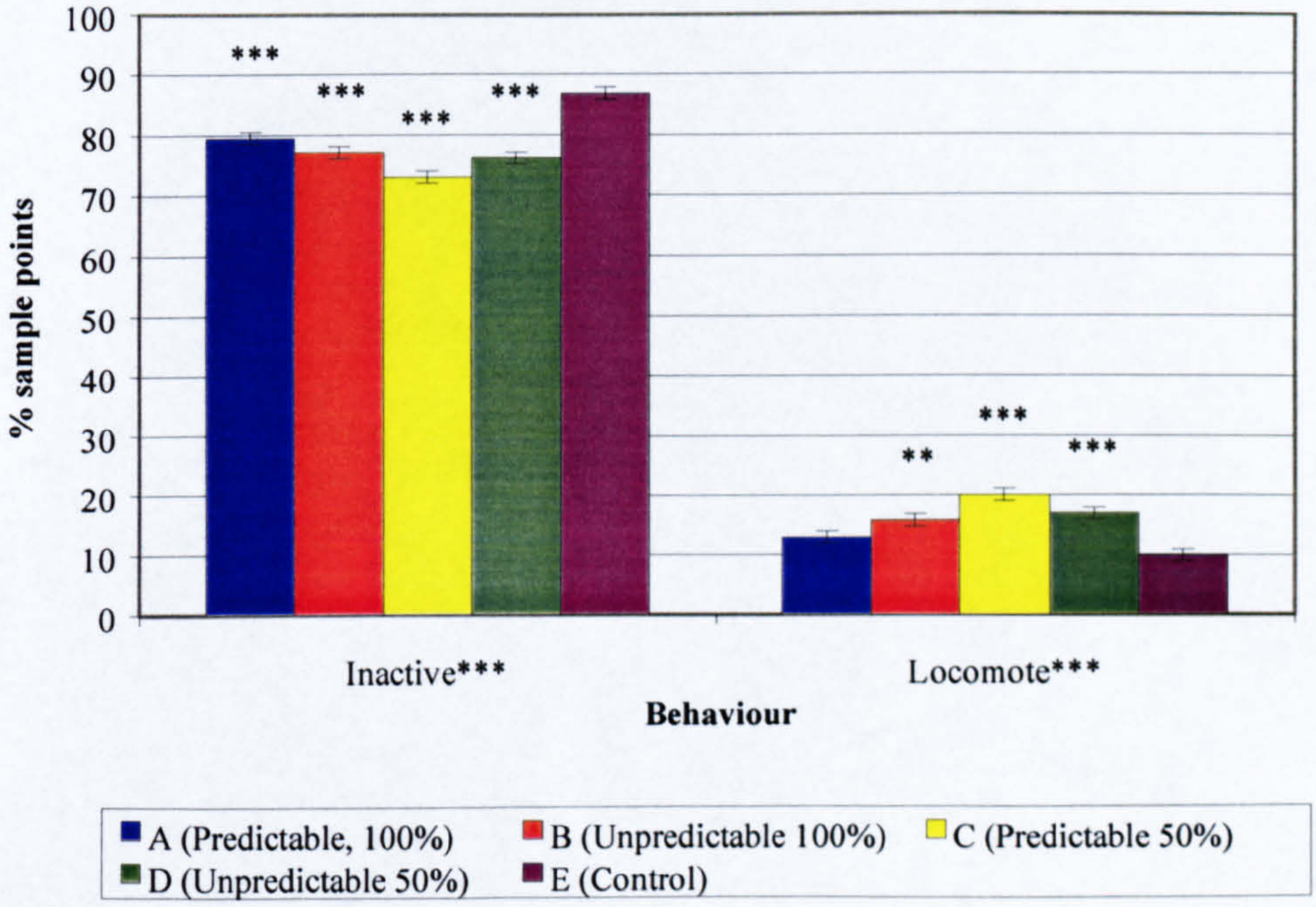
6.3 RESULTS

The effects of each factor (predictability of food delivery, trial period and time period) are initially presented separately. Interactions between the variables are then considered.

6.3.1. Effects of predictability of food delivery on behaviour

Each behavioural category is presented separately. Where a significant main effect was found, results of post-hoc Tukey tests are given.

Figure 6.2 Percentage sample points spent 'inactive' and in 'locomote' in the five study conditions (bars represent Standard Errors)



*p<0.05; **p<0.01; ***p<0.001
Asterisks by x-axis labels indicate significance of between-subject ANOVAs
Asterisks at top of bars indicate significance of differences between respective condition and Control (Condition E)

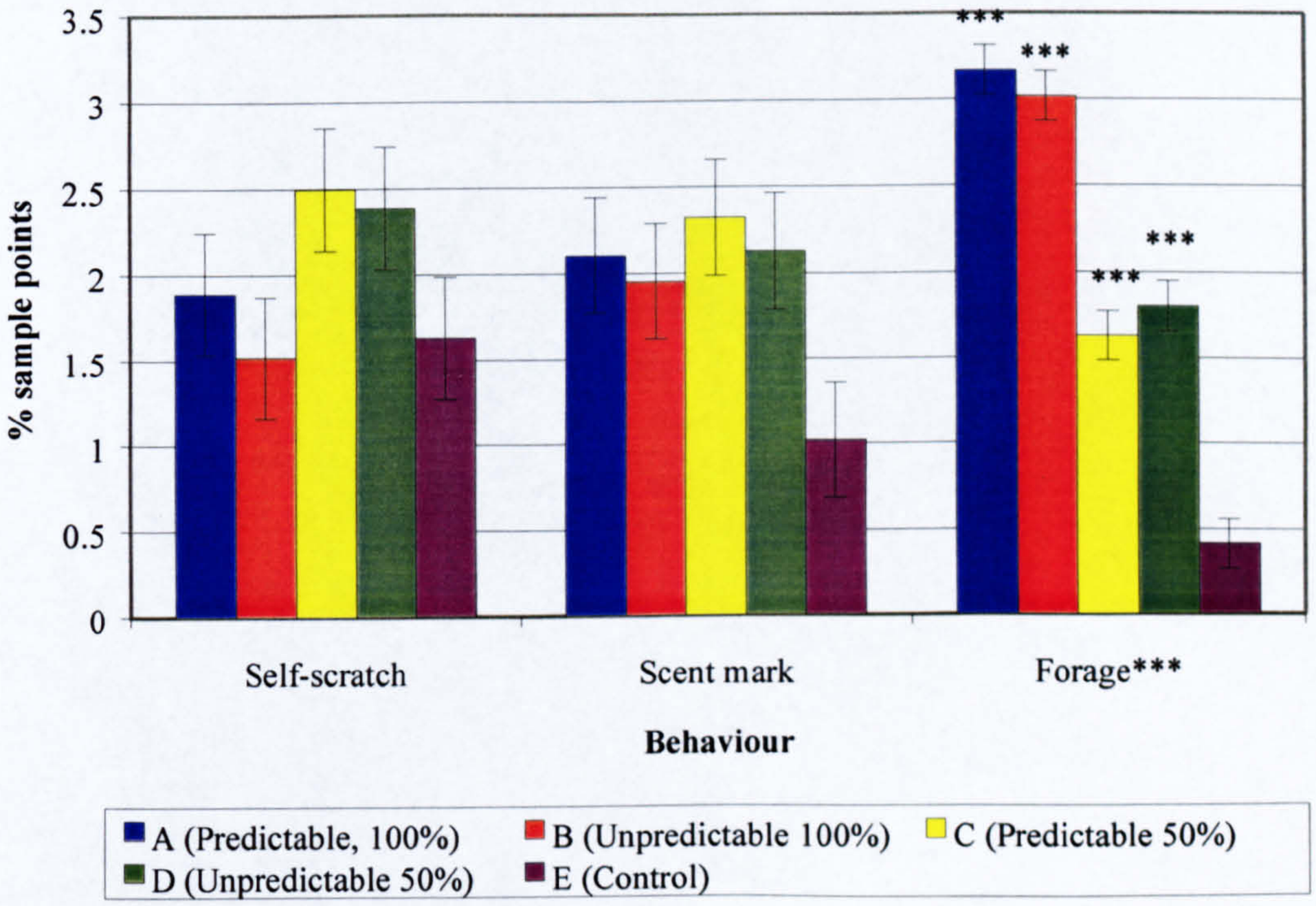
Inactive

There was a significant effect of predictability of food delivery on the total amount of inactivity shown (see Table 6.4). Post-hoc Tukey tests showed that there was significantly less inactivity in each of the four experimental conditions than in the Control condition (Condition E) (see Figure 6.2 and Table 6.5). There was also significantly more inactivity in Condition A than Condition C (see Table 6.5 and Figure 6.2).

Locomote

There was a significant main effect of predictability of feeding on this behaviour (see Table 6.4). There was significantly more locomotion in Conditions B, C and D than in the Control condition (E) (see Table 6.5 and Figure 6.2). There was also significantly more locomotion in Condition C than either Conditions A or B (see Table 6.5).

Figure 6.3 Percentage sample points spent 'self-scratch', 'scent mark' and 'forage' in the five study conditions (bars represent Standard Errors)



*p<0.05; **p<0.01; ***p<0.001
Asterisks by x-axis labels indicate significance of between-subject ANOVAs

Asterisks at top of bars indicate significance of differences between respective condition and Control (Condition E)

Self-scratch and Scent mark

There was no significant main effect of predictability of food delivery on either of these behaviours (see Table 6.4 and Figure 6.3).

Forage

There was a significant main effect of predictability of food delivery on this behaviour (see Table 6.4). There was significantly more foraging in all the experimental conditions than in the Control condition (E) (see Table 6.5 and Figure 6.3). There was also significantly more foraging in Conditions A and B than Conditions C and D (see Table 6.5).

Table 6.4 Results of ANOVAs for effects of predictability of food delivery on all behaviours

Behaviour	**F**	**p**
Inactive	30.29	<0.001***
Locomote	15.92	<0.001***
Self-scratch	1.53	0.22
Scent mark	2.31	0.09
Forage	60.83	<0.001***

All d.f.=4,25 *p<0.05; **p<0.01; ***p<0.001

Table 6.5 Results of post-hoc Tukey tests for effects of predictability of food delivery on behaviour (only behaviours showing a significant main effect of 'predictability' included)

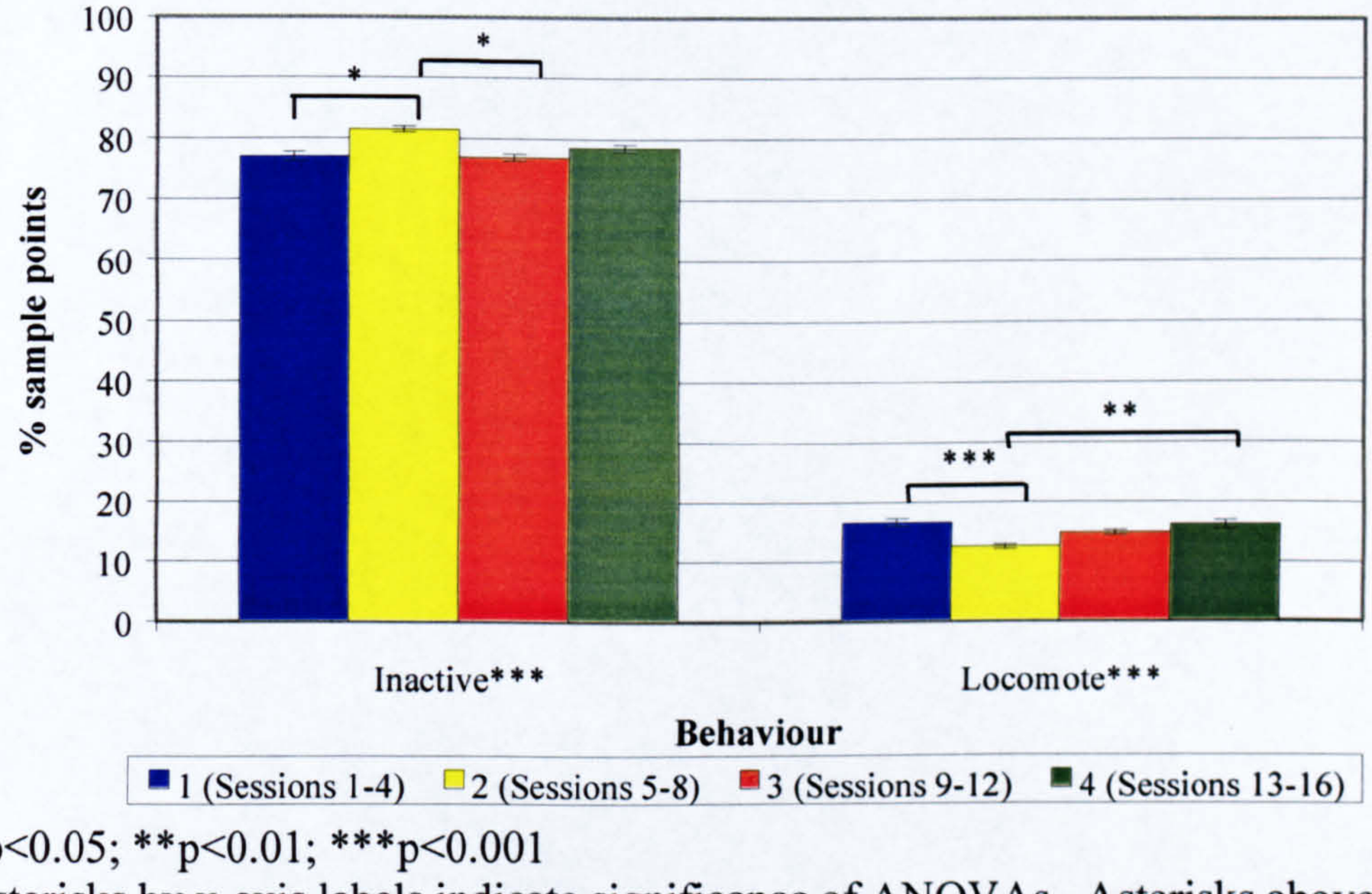
Behaviour	Comparison between conditions	Mean Difference	S. E.	p
Inactive	A-B	2.43	1.35	0.395
	A-C	6.60	1.35	<0.001***
	A-D	3.41	1.35	0.117
	A-E	7.38	1.35	<0.001***
	B-C	4.17	1.35	<0.05*
	B-D	0.98	1.35	0.95
	B-E	9.81	1.35	<0.001***
	C-D	3.19	1.35	0.16
	C-E	13.98	1.35	<0.001***
	D-E	10.79	1.35	<0.001***
Locomote	A-B	2.80	1.37	0.28
	A-C	7.12	1.37	<0.001***
	A-D	3.97	1.37	0.06
	A-E	3.05	1.37	0.20
	B-C	4.32	1.37	<0.05*
	B-D	1.17	1.37	0.91
	B-E	5.86	1.37	<0.01**
	C-D	3.15	1.37	0.18
	C-E	10.18	1.37	<0.001***
	D-E	7.03	1.37	<0.001***
Forage	A-B	0.51	0.20	0.94
	A-C	1.54	0.20	<0.001***
	A-D	1.37	0.20	<0.001***
	A-E	2.76	0.20	<0.001***
	B-C	1.39	0.20	<0.001***
	B-D	1.22	0.20	<0.001***
	B-E	2.60	0.20	<0.001***
	C-D	0.17	0.20	0.91
	C-E	1.22	0.20	<0.001***
	D-E	1.39	0.20	<0.001***

*p<0.05; **p<0.01; ***p<0.001

6.3.2 Effects of trial period on behaviour

Each behavioural category is again presented separately. Where a significant main effect was found, results of post-hoc paired *t*-tests (with the Bonferroni correction) are given.

Figure 6.4 Percentage sample points spent 'inactive' and in 'locomote' in the four trial periods (bars represent Standard Errors)



*p<0.05; **p<0.01; ***p<0.001
Asterisks by x-axis labels indicate significance of ANOVAs. Asterisks above boxes indicate significance of post-hoc pairwise t-tests (following Bonferroni correction).

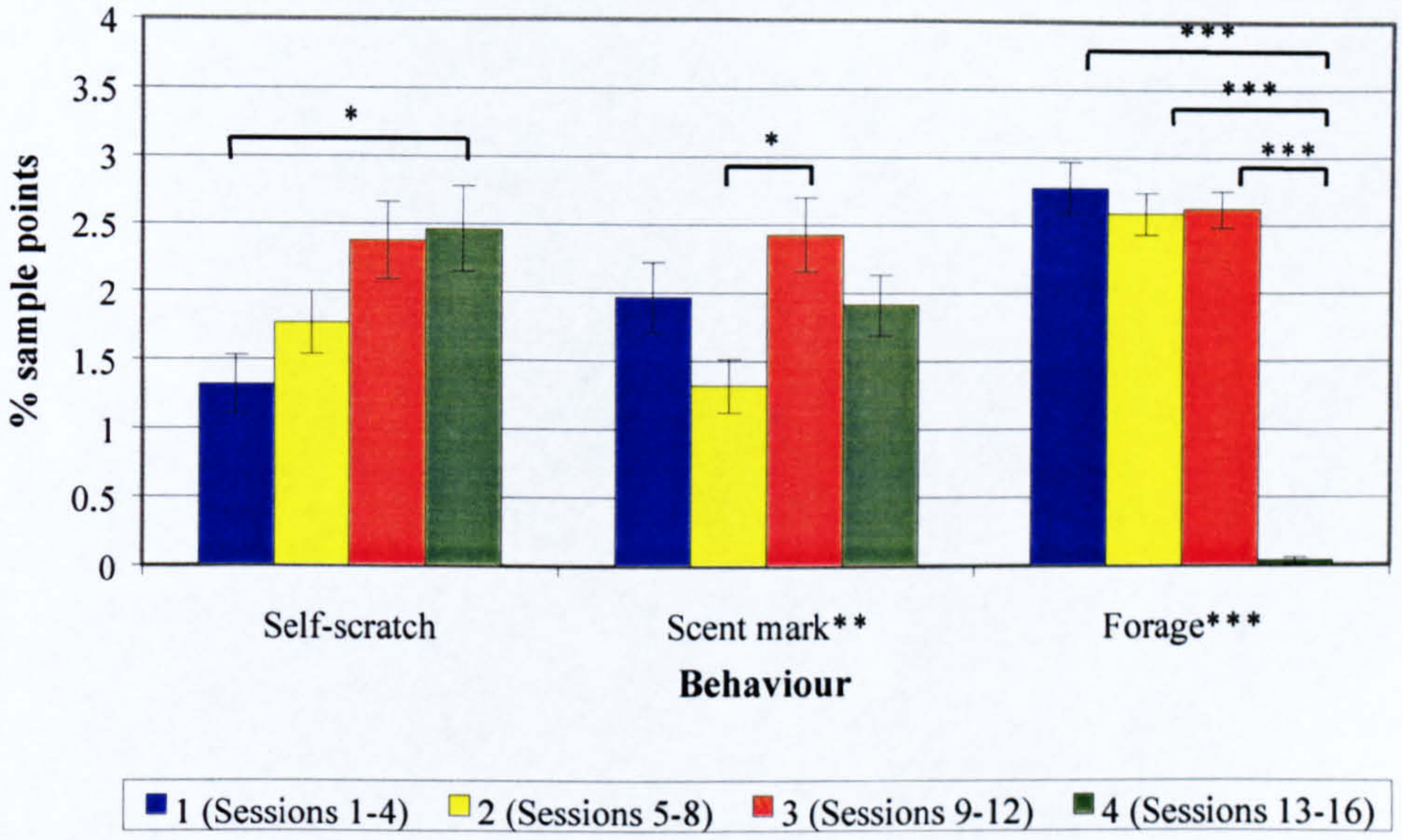
Inactive

There was a significant main effect of trial period on this behaviour (see Table 6.6). There was significantly more inactivity in Trial Period 2 than either Trial Periods 1 or 3 (see Table 6.7 and Figure 6.4).

Locomote

There was a significant main effect of Trial Period on this behaviour (see Table 6.6). There was significantly more locomotion in Trial Periods 1 and 4 than Trial Period 2 (see Table 6.7 and Figure 6.4).

Figure 6.5 Percentage sample points spent 'self-scratch', 'scent mark' and 'forage' in the four trial periods



*p<0.05; **p<0.01; ***p<0.001
Asterisks by x-axis labels indicate significance of ANOVAs. Asterisks above boxes indicate significance of post-hoc pairwise t-tests (following Bonferroni correction).

Self-scratch

There was a significant main effect of Trial Period on this behaviour (see Table 6.6). There was significantly more scratching in Trial Period 4 than Trial Period 1 (see Table 6.7 and Figure 6.5).

Scent mark

There was a significant main effect of Trial Period on this behaviour (see Table 6.6). There was significantly more scent marking in Trial Period 3 than Trial Period 2 (see Table 6.7 and Figure 6.5).

Forage

There was a significant main effect of Trial Period on this behaviour (see Table 6.6). There was significantly more foraging in Trial Periods 1, 2 and 3 than Trial Period 4 (see Table 6.7 and Figure 6.5).

Table 6.6 Results of ANOVAs for effects of trial period on all behaviours

Behaviour	F	p
Inactive	14.67	<0.001***
Locomote	14.23	<0.001***
Self-scratch	5.01	<0.01**
Scent mark	4.61	0.01**
Forage	84.04	<0.001***

All d.f.=2,50 *p<0.05; **p<0.01; ***p<0.001

Table 6.7 Post-hoc *t*-test *t* and p values for mean percentage sample points spent in each behaviour in each trial period (only behaviours showing a significant main effect of 'trial period' included)

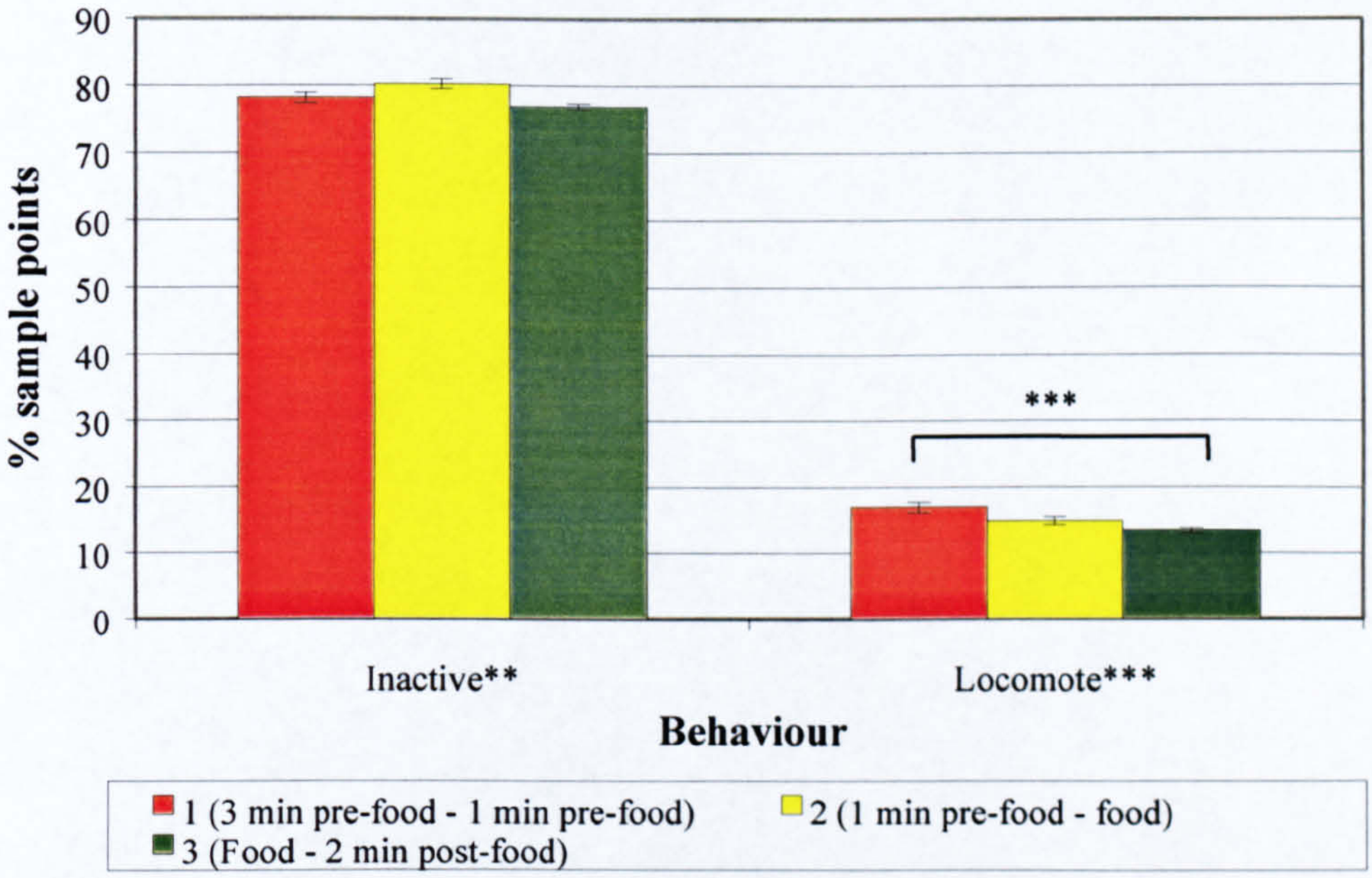
Behaviour	Trial period	*t*	p (uncorrected)	p (following Bonferroni correction)
Inactive	1-2	5.13	<0.001***	<0.001***
	1-3	0.04	0.97	1.00
	1-4	1.30	0.20	1.00
	2-3	4.01	<0.001***	<0.001***
	2-4	2.11	0.04*	0.26
	3-4	0.87	0.39	1.00
Locomote	1-2	5.07	<0.001***	<0.001***
	1-3	1.46	0.16	0.94
	1-4	0.44	0.66	1.00
	2-3	2.82	<0.01**	0.05
	2-4	3.63	<0.001***	<0.01**
	3-4	1.06	0.30	1.00
Self-Scratch	1-2	1.32	0.20	0.59
	1-3	2.66	0.01*	0.08
	1-4	2.95	<0.01**	<0.05*
	2-3	1.92	0.07	0.39
	2-4	1.84	0.08	0.46
	3-4	0.17	0.87	1.00
Scent mark	1-2	2.69	0.01*	0.07
	1-3	1.05	0.30	1.00
	1-4	0.13	0.90	1.00
	2-3	2.83	<0.01**	<0.05*
	2-4	1.46	0.15	0.92
	3-4	1.04	0.31	1.00
Forage	1-2	0.69	0.49	1.00
	1-3	0.81	0.42	1.00
	1-4	9.35	<0.001***	<0.001***
	2-3	0.16	0.88	1.00
	2-4	7.83	<0.001***	<0.001***
	3-4	8.62	<0.001***	<0.001***

All d.f. = 29 *p<0.05; **p<0.01; ***p<0.001

6.3.3 Effects of time in relation to food delivery on behaviour

Each behavioural category is again presented separately. Where a significant main effect was found, results of post-hoc pairwise *t*-tests (with the Bonferroni correction) are given.

Figure 6.6 Percentage sample points spent 'inactive' and in 'locomote' in the three time periods in relation to food delivery (bars represent Standard Errors)



*p<0.05; **p<0.01; ***p<0.001
Asterisks by x-axis labels indicate significance of ANOVAs. Asterisks above boxes indicate significance of post-hoc pairwise t-tests (following Bonferroni correction).

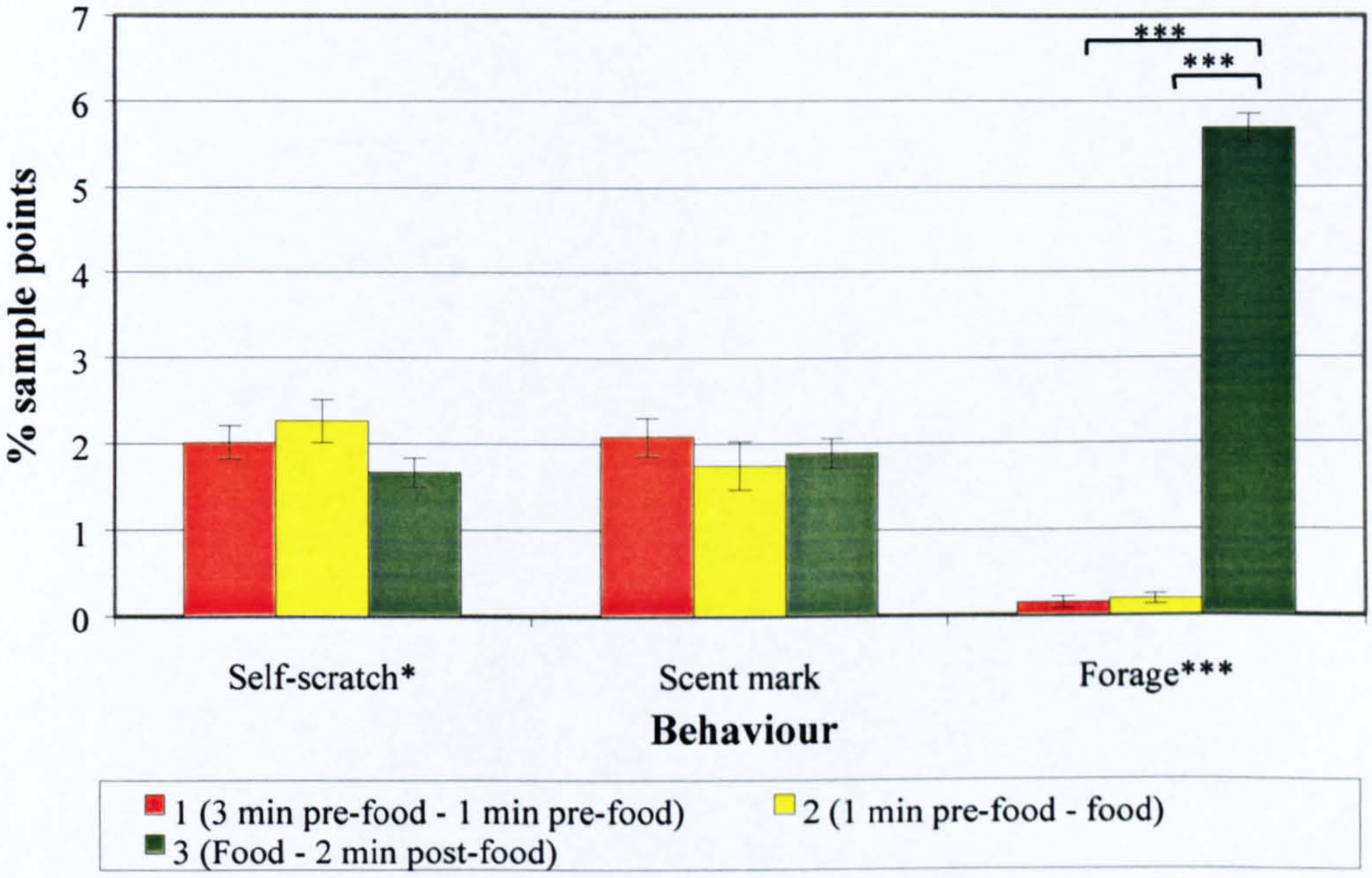
Inactive

There was a significant main effect of Time Period on this behaviour (see Table 6.8). However, none of the individual time periods were significantly different to each other after the Bonferroni correction had been applied to the post-hoc paired *t*-tests (see Table 6.9 and Figure 6.6).

Locomote

There was a significant main effect of Time Period on this behaviour (see Table 6.8). There was significantly more locomotion in Time Period 1 than Time Period 3 (see Table 6.9 and Figure 6.6).

Figure 6.7 Percentage sample points spent in 'self-scratch', 'scent mark' and 'forage' in the three time periods in relation to food delivery (bars represent Standard Errors)



*p<0.05; **p<0.01; ***p<0.001
Asterisks by x-axis labels indicate significance of ANOVAs.

Self-scratch

There was a significant main effect of Time Period on this behaviour (see Table 6.8). However, post-hoc tests showed no significant differences between any of the individual Time Periods (see Table 6.9 and Figure 6.7).

Scent mark

There was no significant main effect of Time Period on this behaviour (see Table 6.9 and Figure 6.7).

Forage

There was a significant main effect of Time Period on this behaviour (see Table 6.8). There was significantly more foraging in Time Period 3 than Time Periods 1 and 2 (see Table 6.9 and Figure 6.7).

Table 6.8 Results of ANOVAs for effects of time in relation to food delivery on all behaviours

Behaviour	F	p
Inactive	8.47	<0.01**
Locomote	11.08	<0.001***
Self-scratch	3.42	<0.05*
Scent mark	0.65	0.53
Forage	982.59	<0.001***

All d.f.=3,75 *p<0.05; **p<0.01; ***p<0.001

Table 6.9 Post-hoc *t*-test *t* and p values for mean percentage sample points spent in each behaviour in each time period in relation to food delivery (only behaviours showing a significant main effect of 'time period' included)

Behaviour	Trial period	*t*	p (uncorrected)	p (following Bonferroni correction)
Inactive	1-2	2.34	<0.05*	0.08
	1-3	1.02	0.32	0.95
	2-3	2.38	<0.05*	0.07
Locomote	1-2	2.52	0.02	0.05
	1-3	4.36	<0.001***	<0.001***
	2-3	1.80	0.08	0.25
Self-scratch	1-2	1.04	0.31	0.93
	1-3	2.24	<0.05*	0.10
	2-3	2.42	<0.05*	0.07
Forage	1-2	0.52	0.61	1.00
	1-3	8.46	<0.001***	<0.001***
	2-3	8.43	<0.001***	<0.001***

All d.f. = 29 *p<0.05; **p<0.01; ***p<0.001

6.3.4 Effects of interactions between the three variables

6.3.4.1 Interactions between 'Trial Period' and 'Predictability of food delivery'

There were significant interactions between 'Trial Period' and 'Predictability of food delivery' on 'Inactive', 'Locomote' 'Self-scratch' and 'Scent mark' (see Table 6.10). Significant interactions are described.

Table 6.10 Results of ANOVAs for interaction between 'Trial Period' and 'Predictability of food delivery' on all behaviours (all trials)

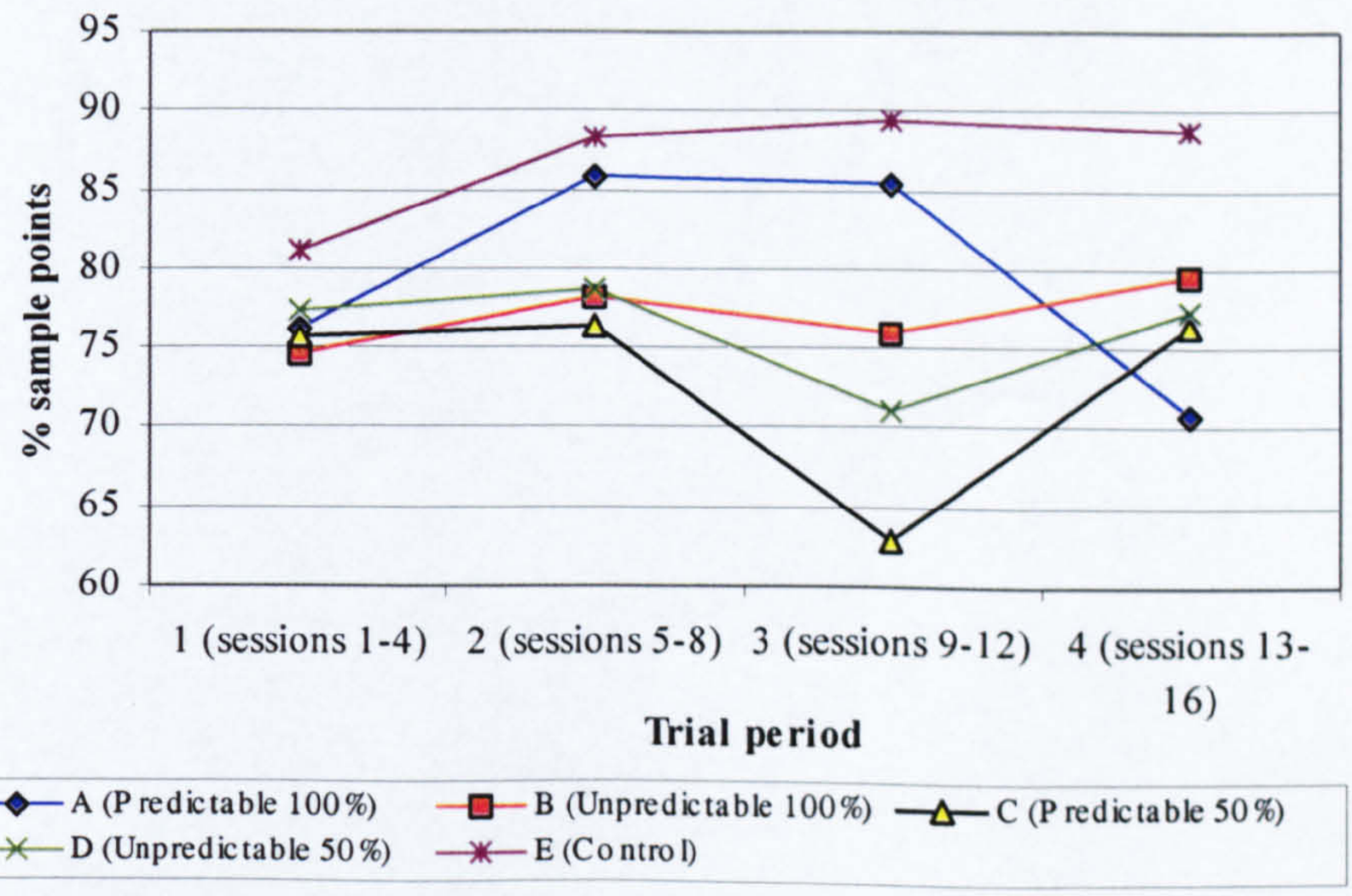
Behaviour	F	p
Inactive	16.82	<0.001***
Locomote	9.73	<0.001***
Self-scratch	3.35	<0.001***
Scent mark	7.04	<0.001***
Forage	1.59	0.11

All d.f.=12,75 *p<0.05; **p<0.01; ***p<0.001

Inactive

There was a similar amount of inactivity in all five conditions in the first trial period. There was a slight increase in the rate of this behaviour in the Control condition in the second trial period, levels remaining similar for the third and forth trial periods. The pattern for inactivity for Condition A was similar to that of the Control condition, except for in Trial Period 4, where rates for Condition A decreased. Levels of this behaviour were fairly similar for Conditions B, C and D, except for in Trial Period 3, where levels fell slightly for Condition D, and more so for Condition C (see Figure 6.8).

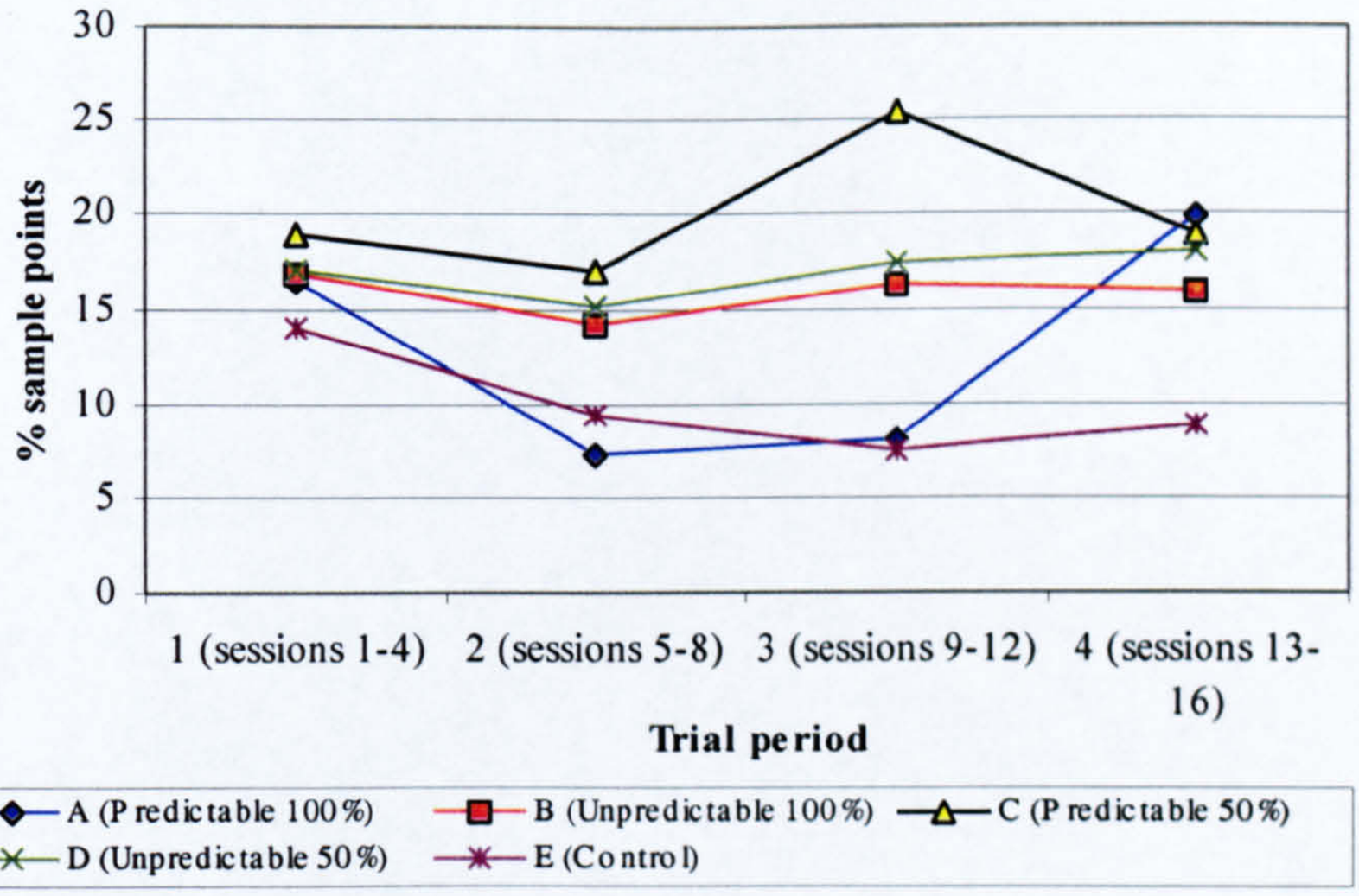
Figure 6.8 Interaction between 'Trial Period' and 'Predictability of food delivery' for 'Inactive'



Locomote

The pattern seen for 'locomote' was the inverse of that seen for 'inactive'. Rates of locomotion were similar for all four conditions for Trial Period 1. At Trial Period 3, locomotion was lowest in Conditions A and E, intermediate for Conditions B and D and highest for Condition C. However, in Trial Period 4, levels for all four experimental conditions were similar, with animals in the Control condition showing lower rates of locomotion. Conditions A and E showed similar rates of locomotion for Trial Periods 1-3, but animals in Condition A showed an increase in locomotion for Trial Period 4 that was not seen in the Control condition (see Figure 6.9).

Figure 6.9 Interaction between 'Trial Period' and 'Predictability of food delivery' for 'Locomote'

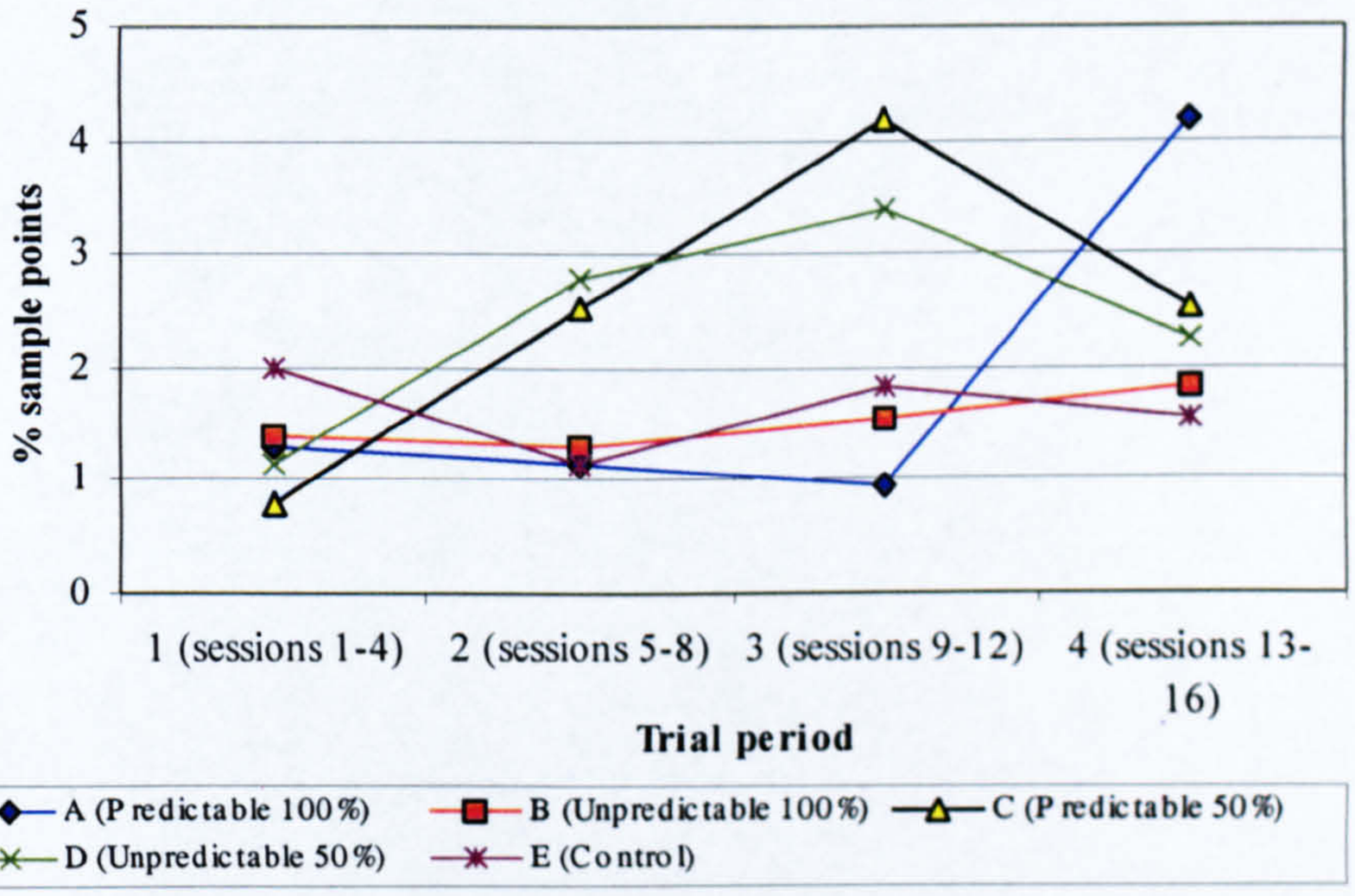


Self-Scratch

Rates of this behaviour were similar for all conditions at Trial Period 1. However, at Trial Periods 2 and 3, rates were higher for Conditions C and D than for Conditions A, B and the Control condition. Rates of scratching remained constant and similar over all four trial periods for Conditions B and the Control condition. Condition A animals displayed rates similar to these for the first three

trial periods, but rates increased sharply for this condition for the final trial period. Rates for Conditions C and D were also similar throughout the study period, increasing over the first three trial periods and decreasing at Trial Period 4. At Trial Period 4, rates for Conditions B, C, D and the Control were similar, but Condition A animals showed more scratching for this trial period (see Figure 6.10).

Figure 6.10 Interaction between 'Trial Period' and 'Predictability of food delivery' for 'Self-scratch'

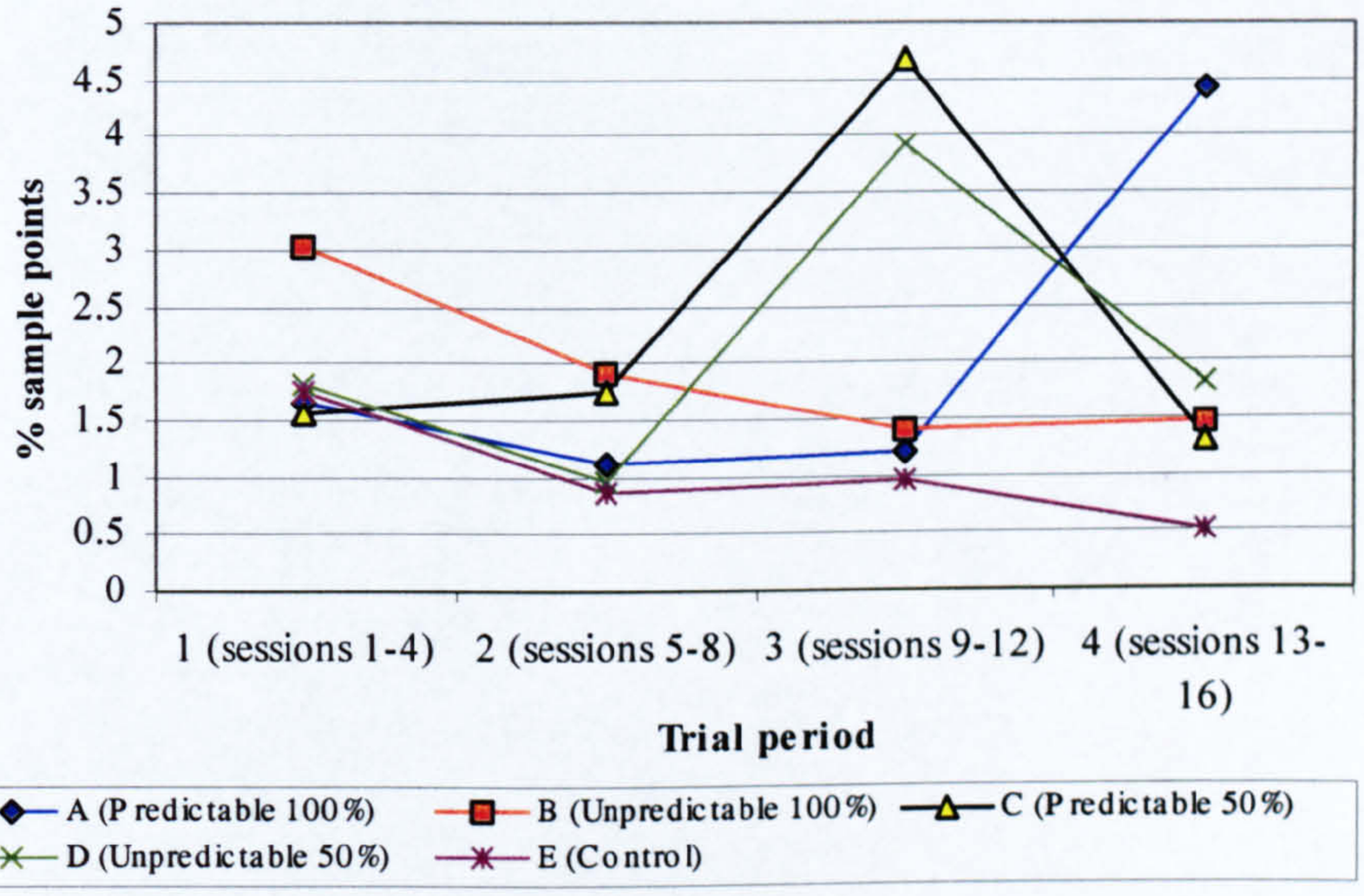


Scent mark

The pattern seen for scent marking was similar to that of scratching. Rates of scent marking were similar for Conditions A, C, D and E for the first trial period. There was more scent marking for Condition B in this period, however. By Trial Period 2, rates of scent marking were similar for all conditions. However, at Trial Period 3, there was more scent marking in Conditions C and D, with the other three conditions showing fairly low rates, similar to those seen in Trial Period 2. At Trial Period 4, however, rates in Conditions C and D had fallen to levels similar to those of Conditions B and E, whereas Condition A animals showed a marked increase in scent marking. As was the case for scratching, Condition A animals showed a

pattern similar to that shown by Control animals, except for in Trial Period 4, where rates increased for Condition A, but not Control, animals (see Figure 6.11).

Figure 6.11 Interaction between 'Trial Period' and 'Predictability of food delivery' for 'Scent mark'



6.3.4.2 Interactions between 'Time Period' and 'Predictability of food delivery'

There were significant interactions between 'Time Period' and 'Predictability of food delivery' on 'Inactive', 'Locomote' and 'Scent mark' (see Table 6.11). Significant interactions are described below.

Table 6.11 Results of ANOVAs for interaction between 'Time Period' and 'Predictability of food delivery' on all behaviours

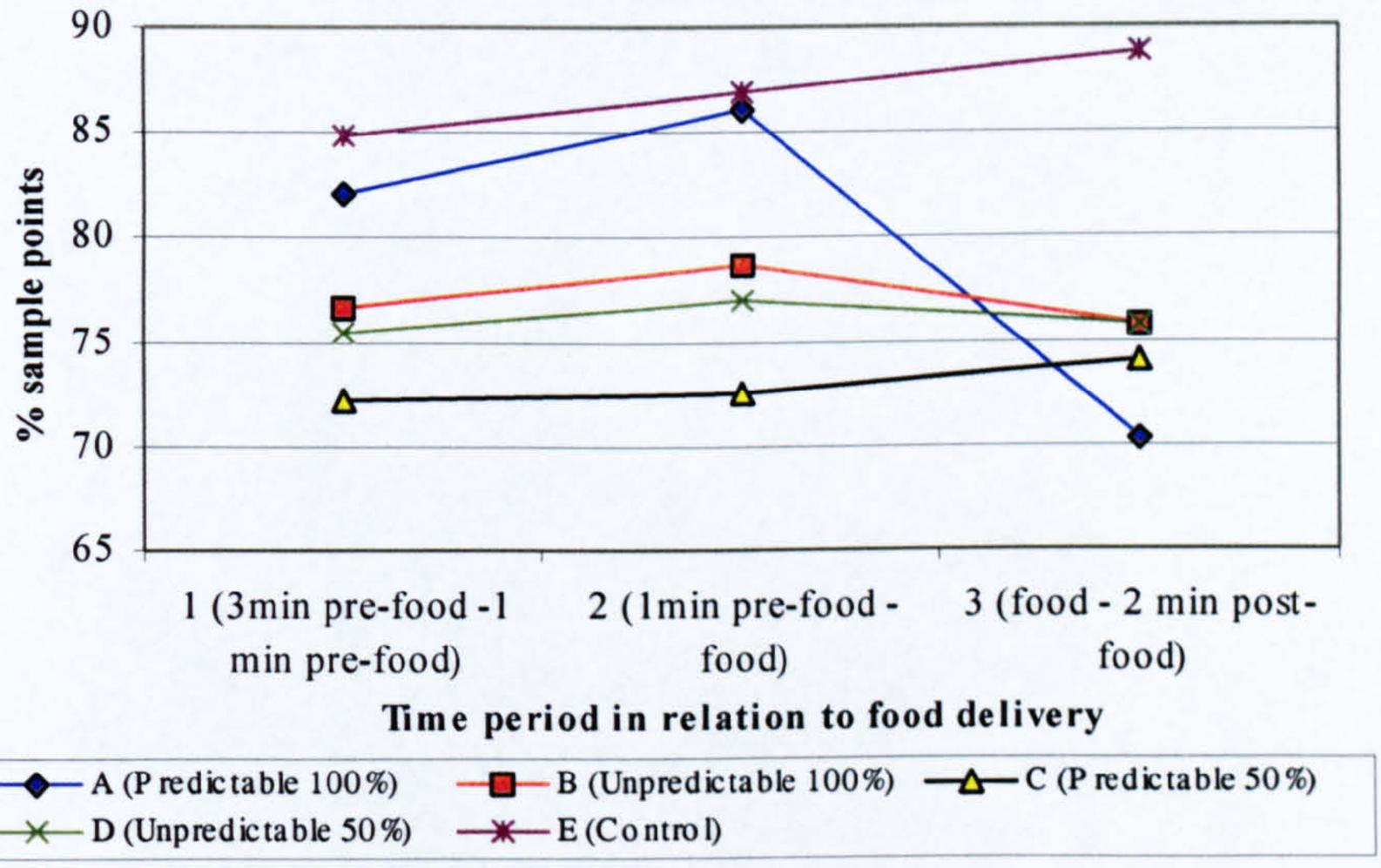
Behaviour	F	p
Inactive	9.42	<0.001***
Locomote	2.30	<0.05*
Self-scratch	0.27	0.97
Scent mark	3.13	<0.01**
Forage	0.76	0.64

All d.f.= 8,50 *p<0.05; **p<0.01; ***p<0.001

Inactive

Rates of this behaviour were similar across all three time periods for Conditions B, C, D and E. Rates in all three time periods were greater in the Control condition than in Conditions B, C and D. However, Condition A animals showed similar rates as Control animals for the first two time periods, but a marked decrease in rates of the behaviour in Time Period 3 (see Figure 6.12).

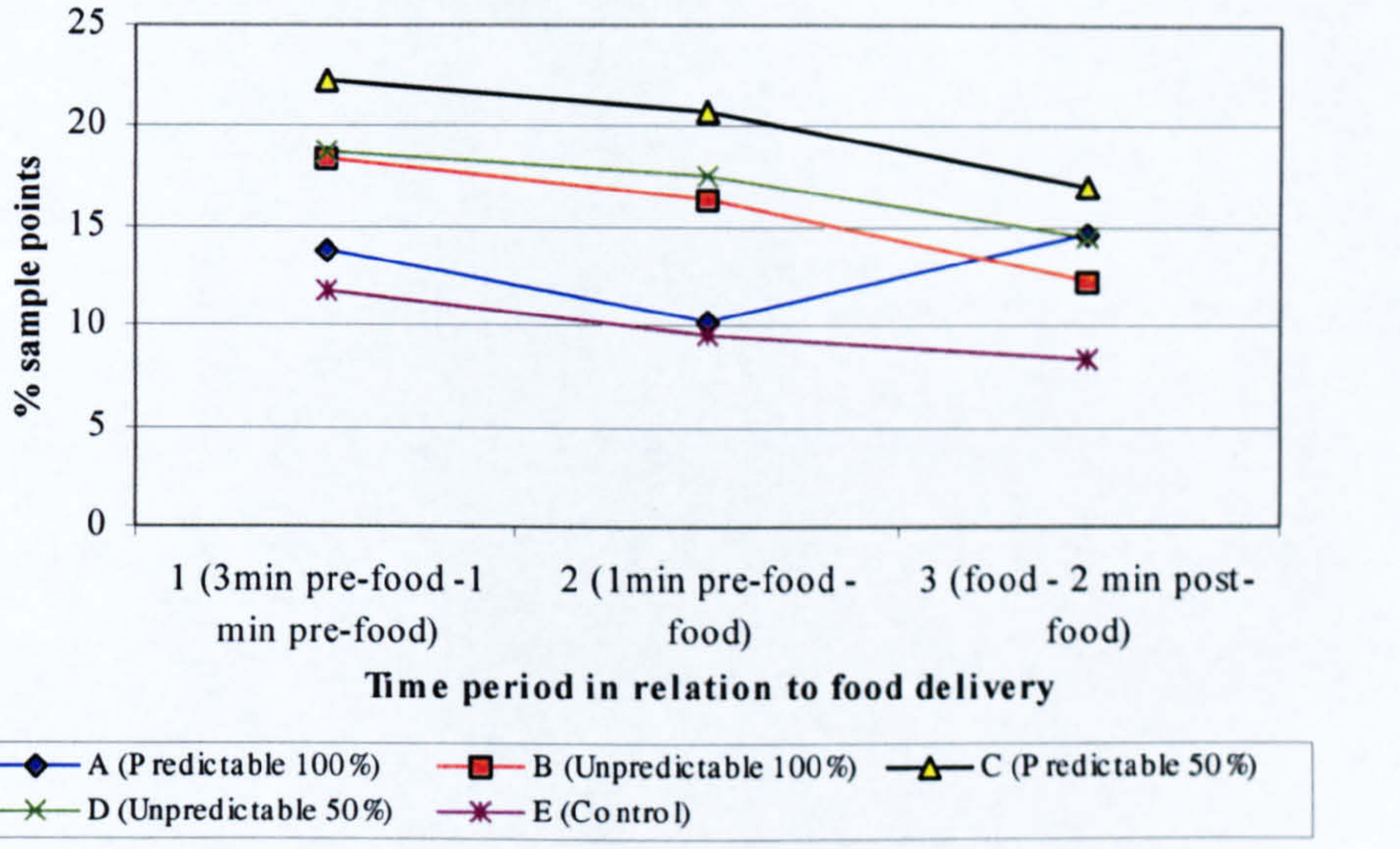
Figure 6.12 Interaction between 'Time Period' and 'Predictability of food delivery' for 'Inactive'



Locomote

There was a slight decrease in this behaviour over Time Periods 1, 2 and 3 for Conditions B, C, D and E. Rates in Condition A were similar to those of the Control condition for Time Periods 1 and 2, but increased for Time Period 3 (see Figure 6.13).

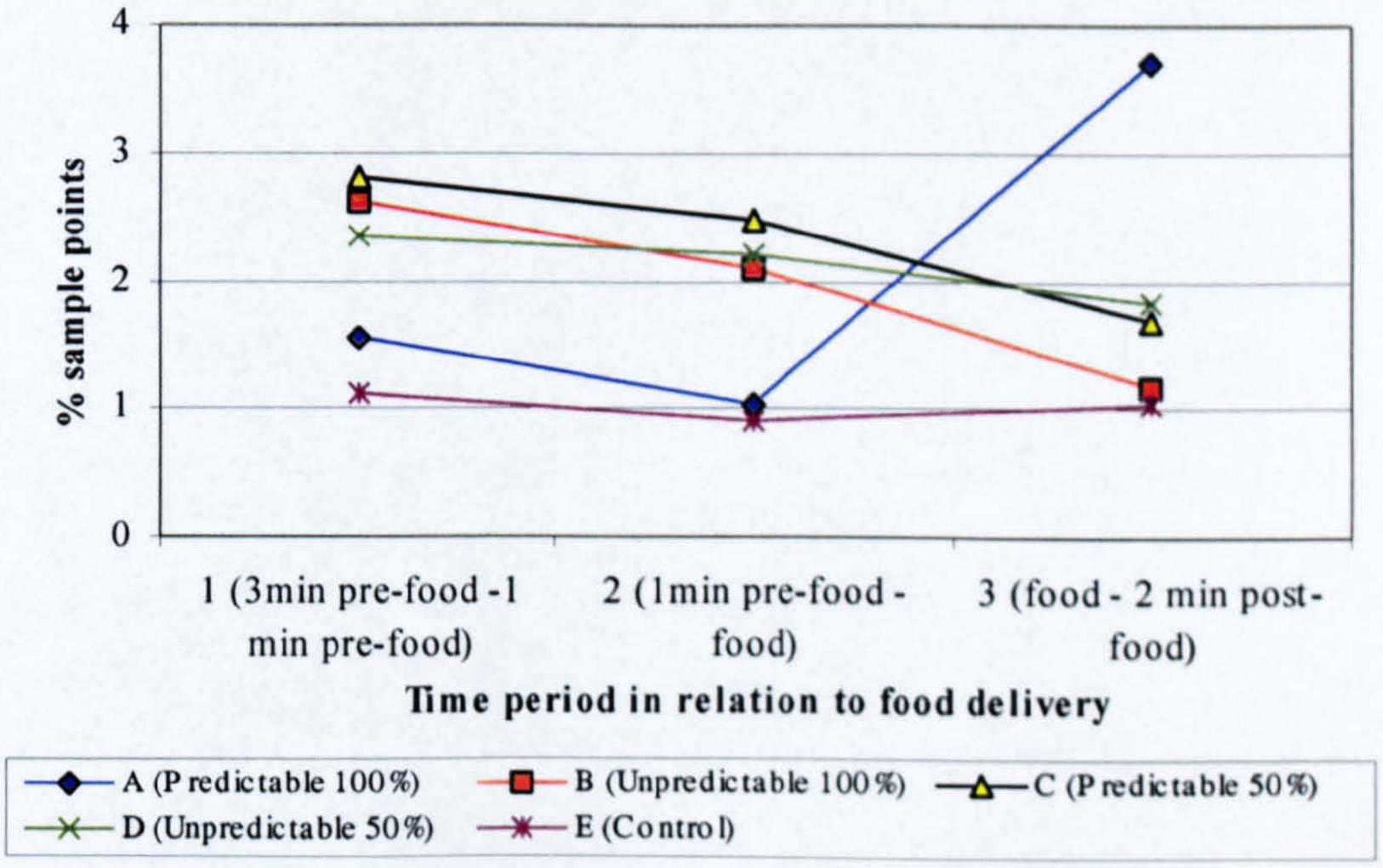
Figure 6.13 Interaction between 'Time Period' and 'Predictability of food delivery' for 'locomote'



Scent mark

Rates of scent marking were fairly constant for the Control condition over the three time periods. There was a slight decrease in the behaviour over the three time periods in Conditions B, C and D. Rates in Condition A were similar to those in the Control condition for the first two time periods. However, in Time Period 3, rates in Condition A increased to the highest level of the five conditions (see Figure 6.14).

Figure 6.14 Interaction between 'Time Period' and 'Predictability of food delivery' for 'Scent mark'



6.3.4.3 Interactions between 'Trial Period' and 'Time Period'

There were significant interactions between 'Trial Period' and 'Time Period' on 'Locomote' and 'Scent mark' (see Table 6.12). Significant interactions are described below.

Table 6.12 Results of ANOVAs for interaction between 'Trial Period' and 'Time Period' on all behaviours

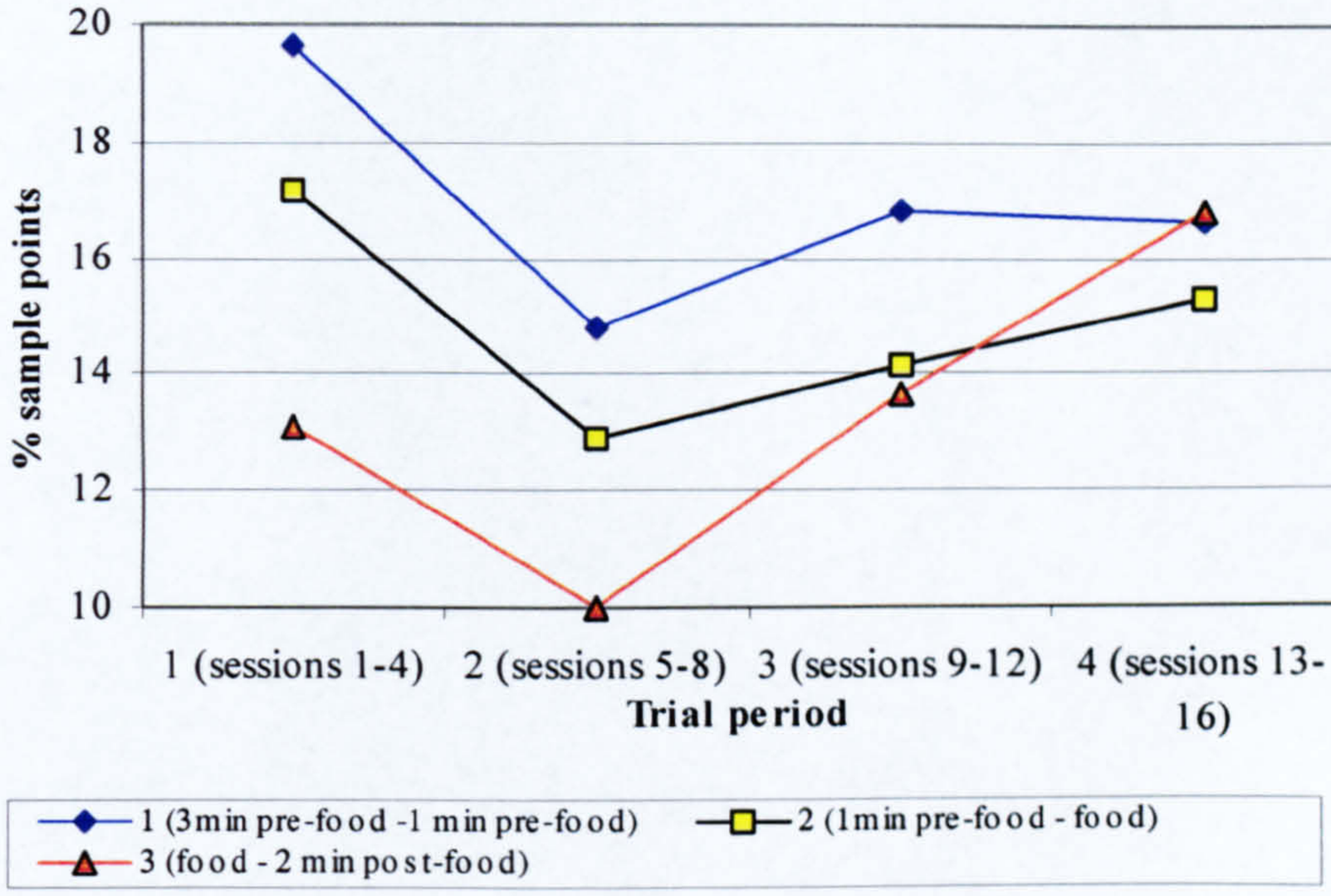
Behaviour	F	p
Inactive	0.60	0.74
Locomote	3.83	<0.001***
Self-scratch	1.79	0.10
Scent mark	5.16	<0.001***
Forage	0.39	0.88

All d.f.= 8,50 *p<0.05; **p<0.01; ***p<0.001

Locomote

At Trial Periods 1 and 2, the most locomotion occurred at Time Period 1 and the least at Time Period 3, with Time Period 2 intermediate between the two. At Time Period 3, the highest levels of locomotion again occurred at Time Period 1, with lower levels seen at Time Periods 2 and 3, but rates at the last two time periods were similar to each other. At Time Period 4, rates were similar at all three time periods. Decreases in rates of locomotion were seen for all three time periods at Trial Period 2 compared with values at the first trial period. An increase was seen for Time Period 1 at Trial Period 3, but this levelled off at Trial Period 4. Increases in locomotion over the last two trial periods were seen at Time Periods 2 and 3 (see Figure 6.15).

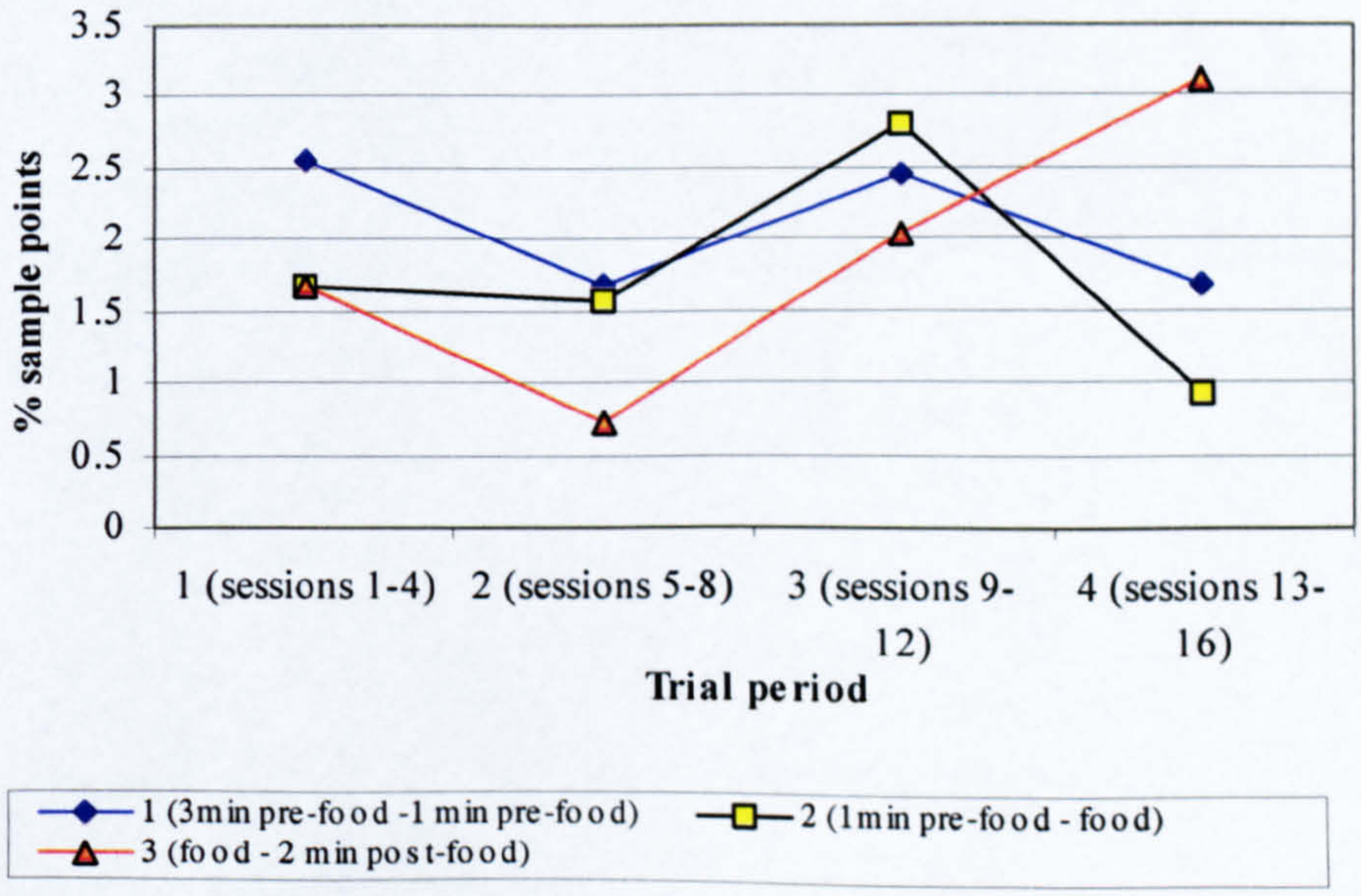
Figure 6.15 Interaction between 'Time Period' and 'Trial Period' for 'Locomote'



Scent Mark

The amount of scent marking seen for Time Period 1 peaked in Trial Periods 1 and 3, with a lower rate shown in Trial Periods 2 and 4. Rates for Time Period 2 were a constant, intermediate, level for Trial Periods 1 and 2, with a peak seen at Trial Period 3 and a nadir at Trial period 4. An intermediate amount of scent marking was seen for Time Period 3 at Trial Periods 1 and 3, with a nadir at Trial Period 2 and a peak at Trial Period 4 (see Figure 6.16).

Figure 6.16 Interaction between 'Time Period' and 'Trial Period' for 'Scent mark'



6.3.4.4 Three-way interactions between 'Predictability of food delivery', 'Trial Period' and 'Time Period'

There were significant interactions between 'Predictability of food delivery', 'Trial Period' and 'Time Period' on 'Inactive', 'Locomote' and 'Scent mark' (see Table 6.13). Significant interactions are described below.

Table 6.13 Results of ANOVAs for interaction between signal, 'Trial Period' and 'Predictability of food delivery' on all behaviours (all trials)

Behaviour	F	p
Inactive	1.76	<0.05*
Locomote	2.39	<0.001***
Self-scratch	1.49	0.08
Scent mark	2.59	<0.001***
Forage	0.46	0.99

All d.f.= 24,150 *p<0.05; **p<0.01; ***p<0.001

Inactive

Rates of inactivity were similar across all three time periods for the Control condition. Rates of this behaviour for Control animals were constant across Trial Periods 2, 3 and 4 but lower in Trial Period 1. For Trial Periods 1-3, Condition A animals showed similar rates to the Control animals for the first two time periods, but a decrease in the behaviour in Time Period 3. In Trial Period 4, rates were lower across all four time periods, with the decrease at Time Period 3 resulting in the lowest rate of the behaviour seen for any condition in the study. Rates of inactivity were relatively unchanged across all time and trial periods for Conditions B and D. Rates for Condition C were similarly unchanged across all time periods, but lower in Trial Period 3 than the other trial periods (see Figure 6.17).

Locomote

Rates of locomotion were fairly constant across all three time periods, in all trial periods for the Control condition. Rates were slightly higher for the Control

condition in the first trial period than in the other three trial periods. Rates for Condition A animals were similar to those of the Control condition in all instances, except for in Trial period 4, when they were slightly higher than the Control for Time Periods 1 and 2, and increased sharply to a peak in Time Period 3. Rates for Conditions B and D were broadly similar and intermediate throughout the study period. Rates for Condition C were slightly higher than for the other conditions in Trial Periods 2 and 3. In Trial Period 3, rates of locomotion for Condition C animals were lowest in Time Period 2, whereas the lowest rates for this condition in the other three trial periods were seen in Time Period 3 (see Figure 6.18).

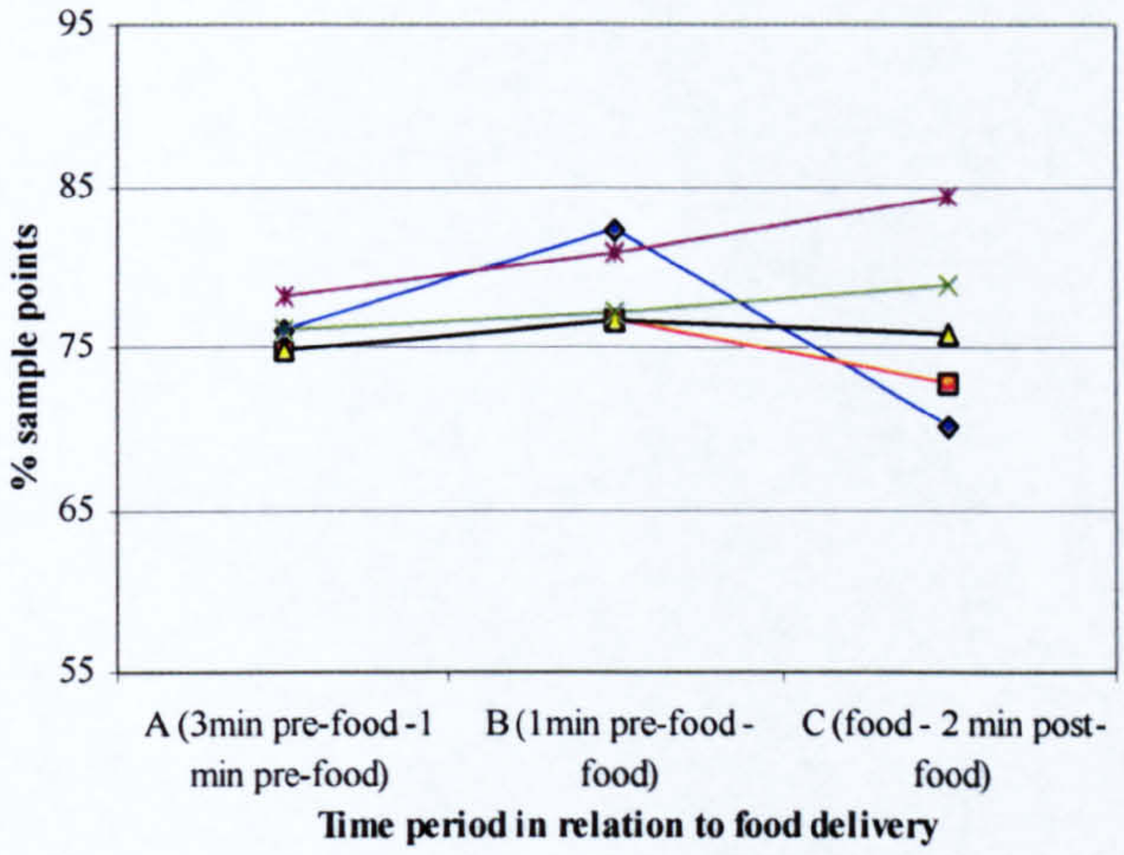
Scent Mark

Rates of scent marking were fairly low, and similar across all time and trial periods for the Control condition. Rates shown by animals in Condition A were similar to those of Control animals in all trial periods and for all time periods, except for Time Period 3 in Trial Period 4. Here a very sharp increase in the amount of scent marking was seen, resulting in a peak considerably greater than was seen for any other condition in the study.

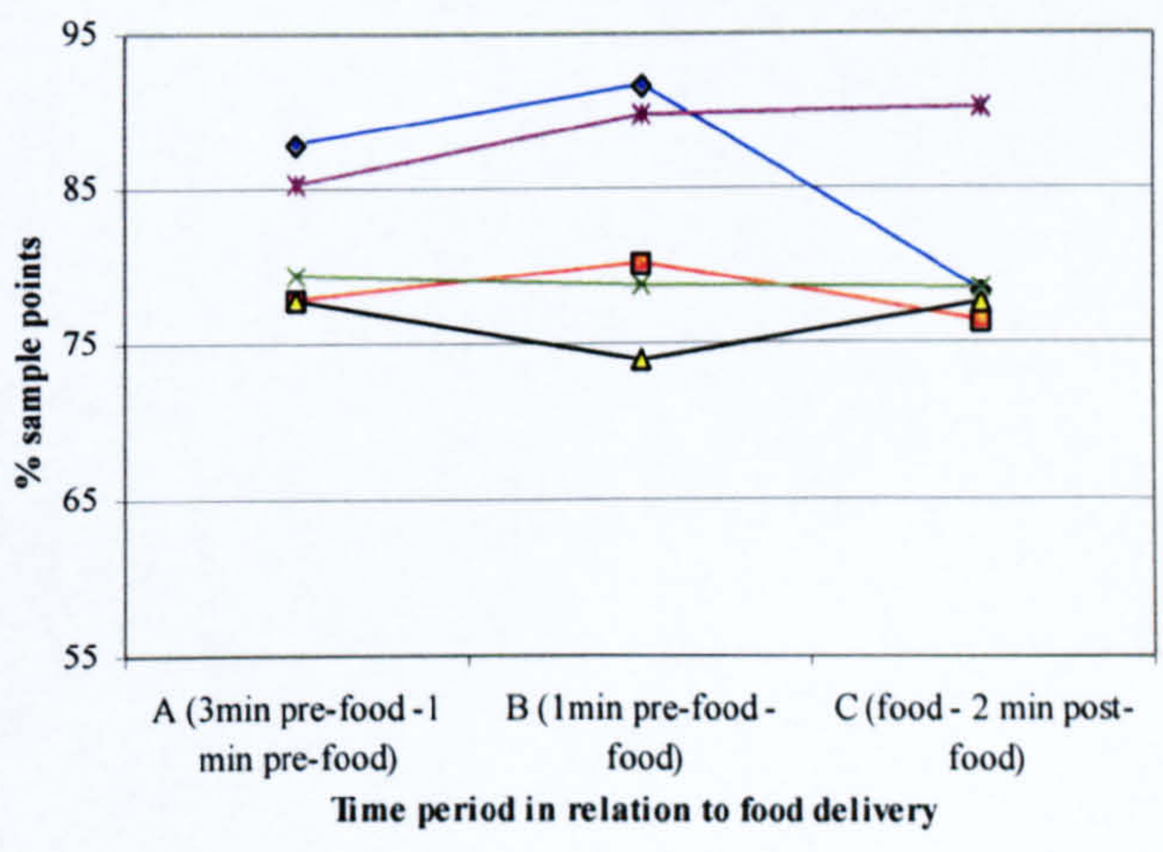
Condition B animals showed similar rates across all time and trial periods, except for Time Period 1 in Trial Period 1, when there was slightly more scent marking than elsewhere. Conditions C and D showed similar patterns to each other, with rates similar across all time periods. All trial periods were also similar, except for Trial Period 3 in which rates were higher than in the other three periods (see Figure 6.19). Rates were also slightly higher for Conditions C and D in Time Period 2 (*i.e.* directly before the food was delivered) than in Time Periods 1 and 3, but only in Trial Period 3.

Figure 6.17 Interaction between 'Time Period' and 'Predictability of food delivery' for each of the four trial periods for 'Inactive'

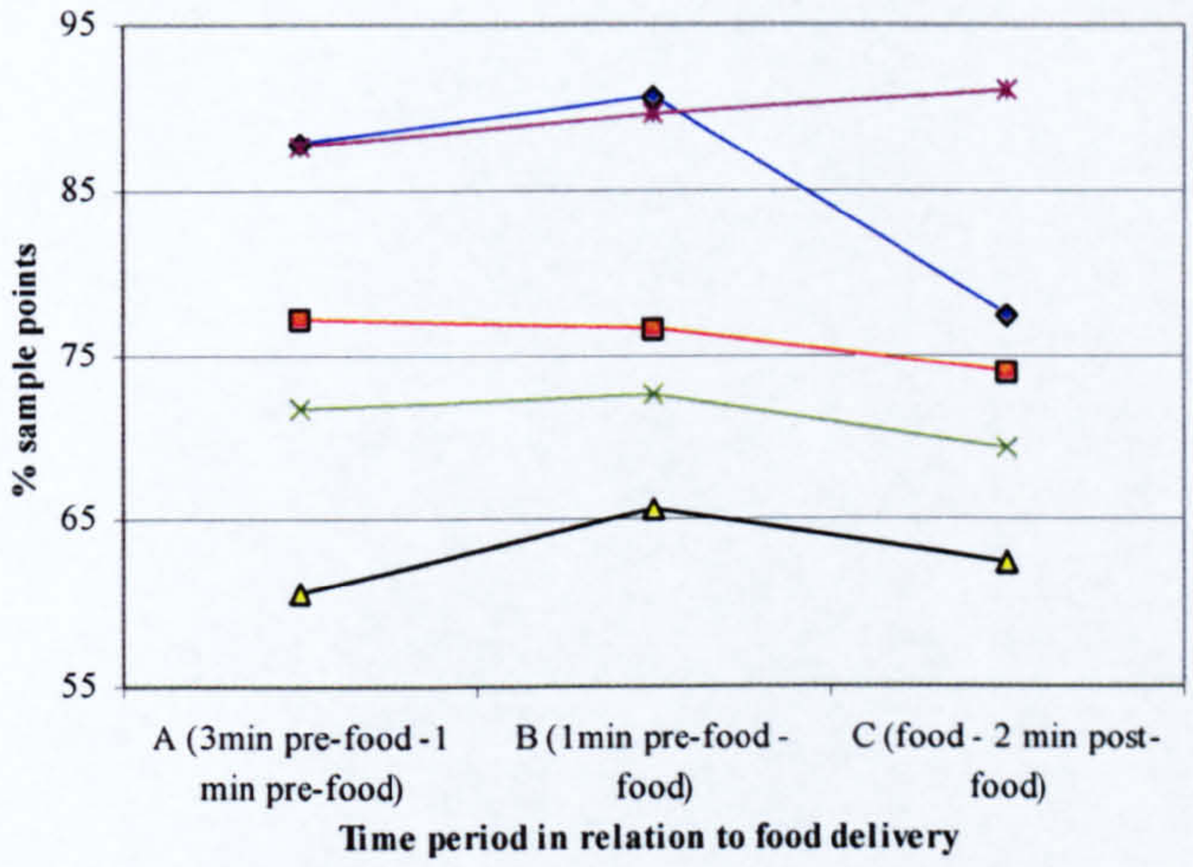
a. Trial period 1 (sessions 1-4)



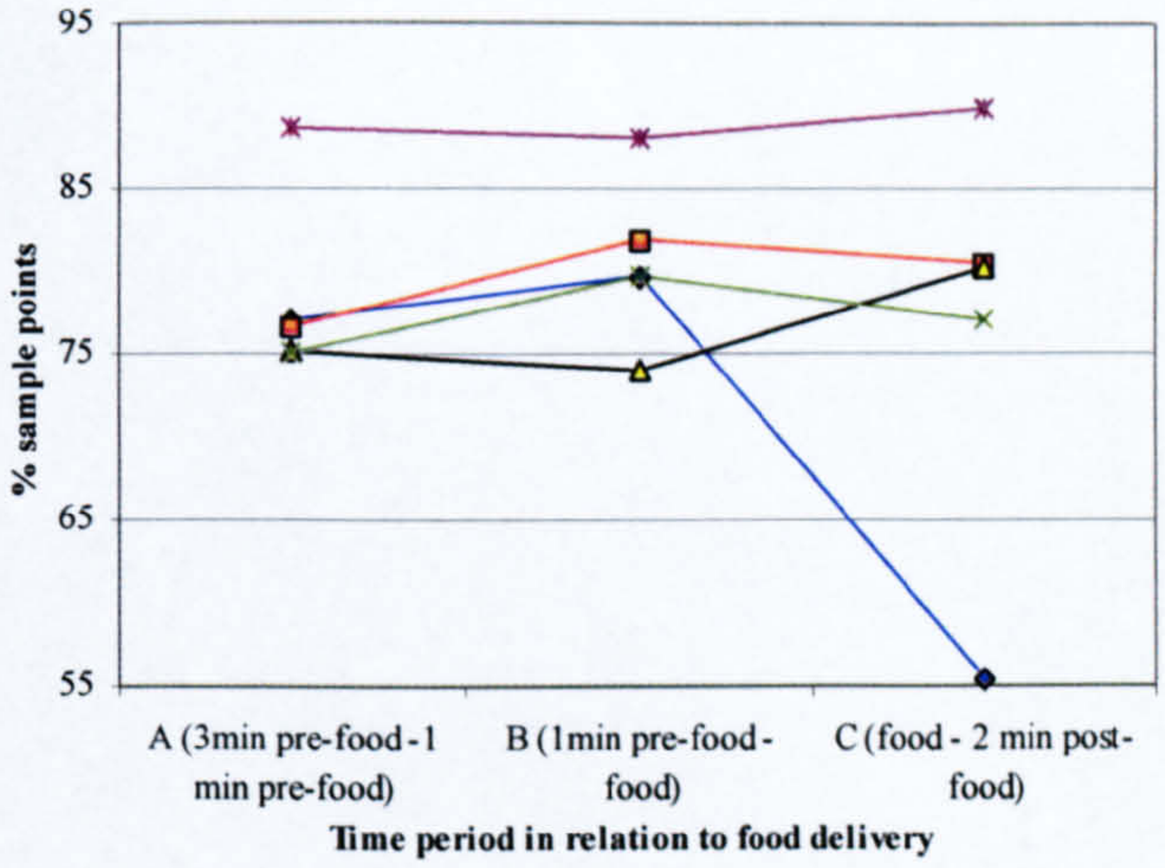
b. Trial period 2 (sessions 5-8)



c. Trial period 3 (sessions 9-12)



d. Trial period 4 (sessions 13-16)



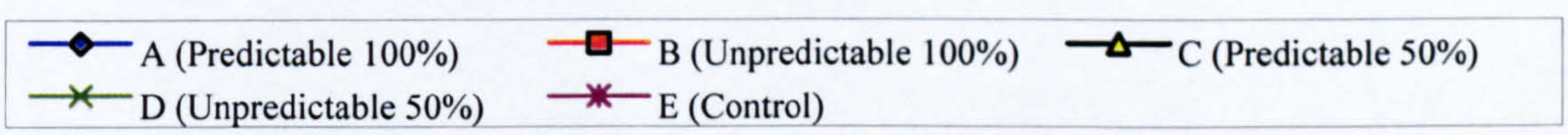
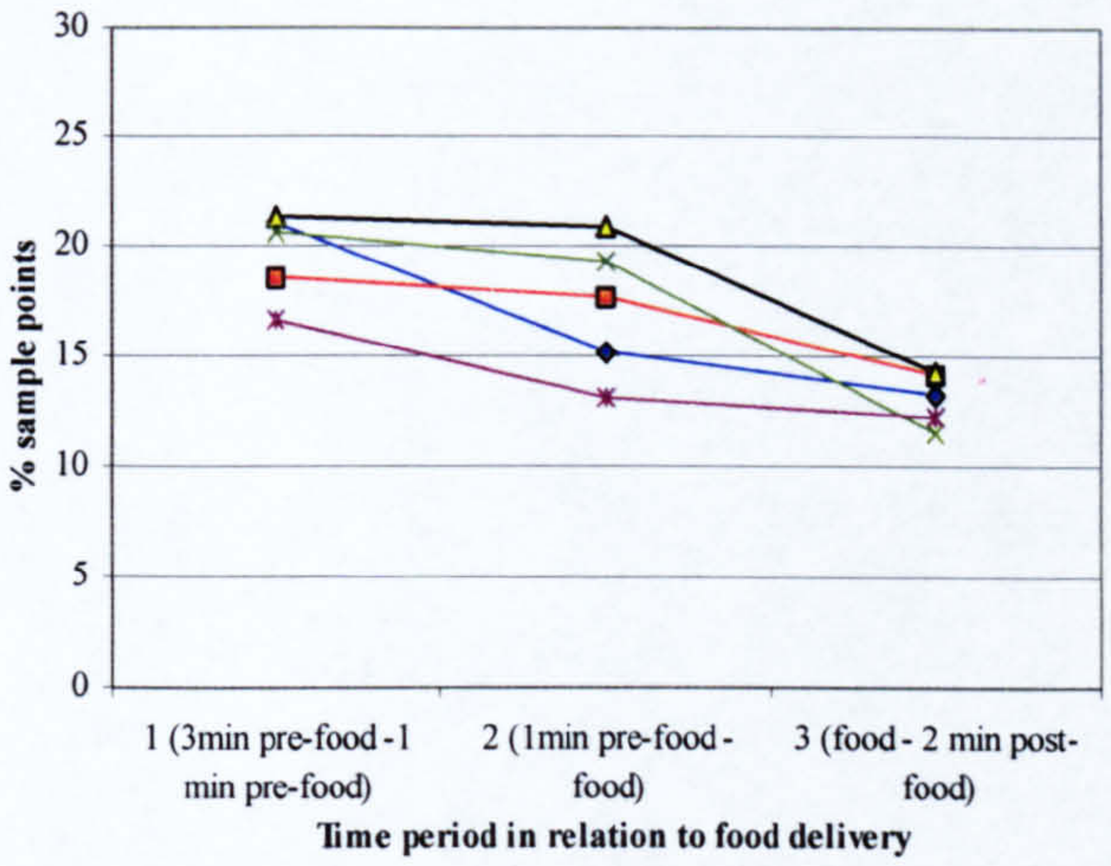
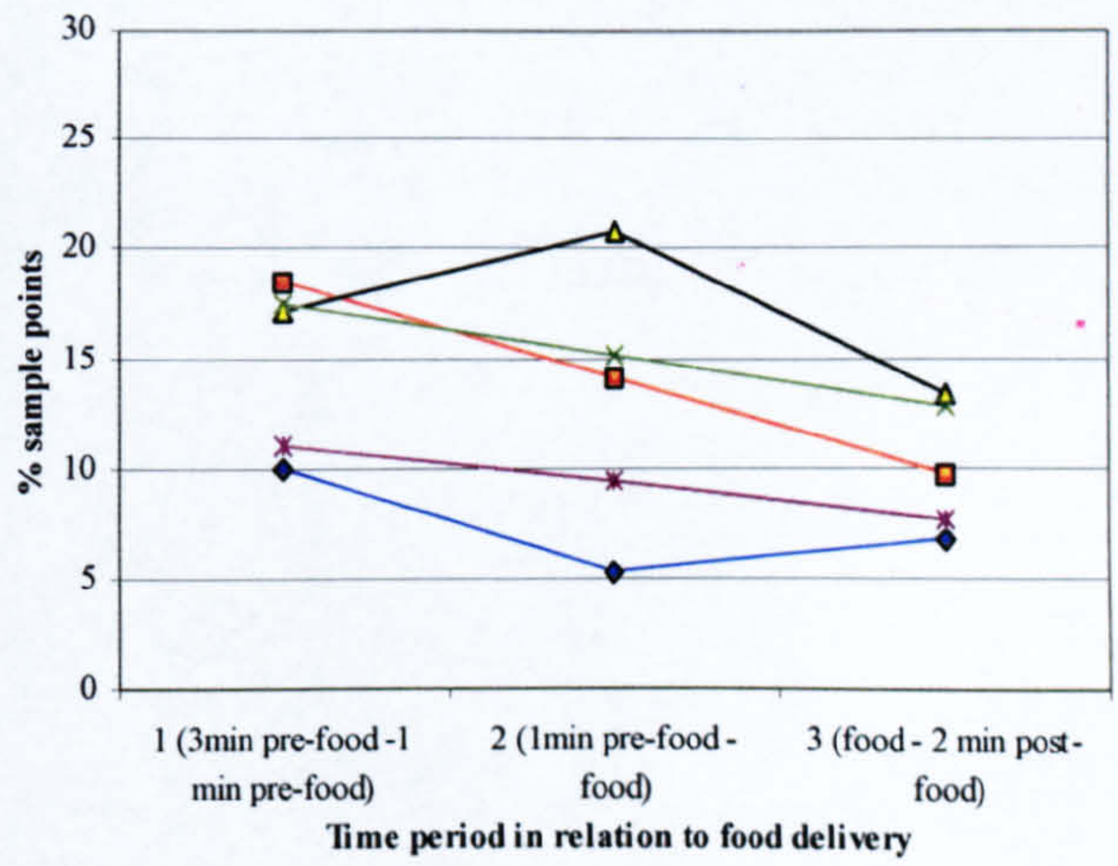


Figure 6.18 Interaction between 'Time Period' and 'Predictability of food delivery' for each of the four trial periods for 'Locomote'

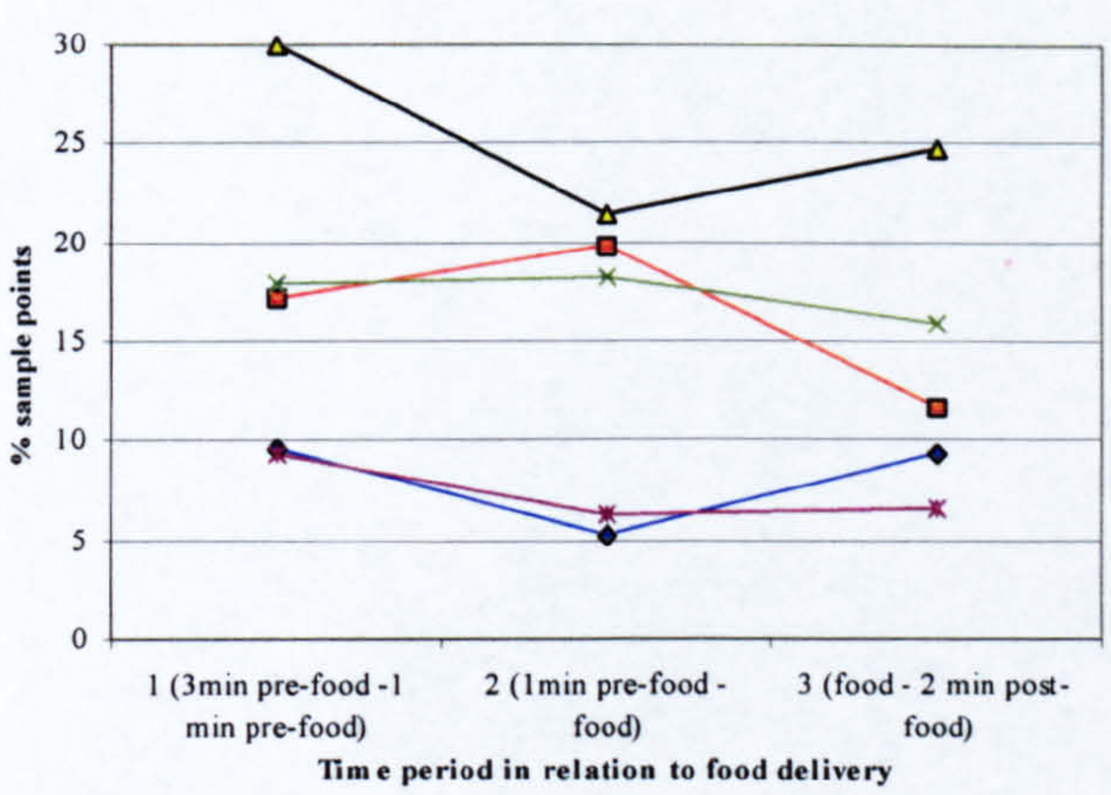
a. Trial period 1 (sessions 1-4)



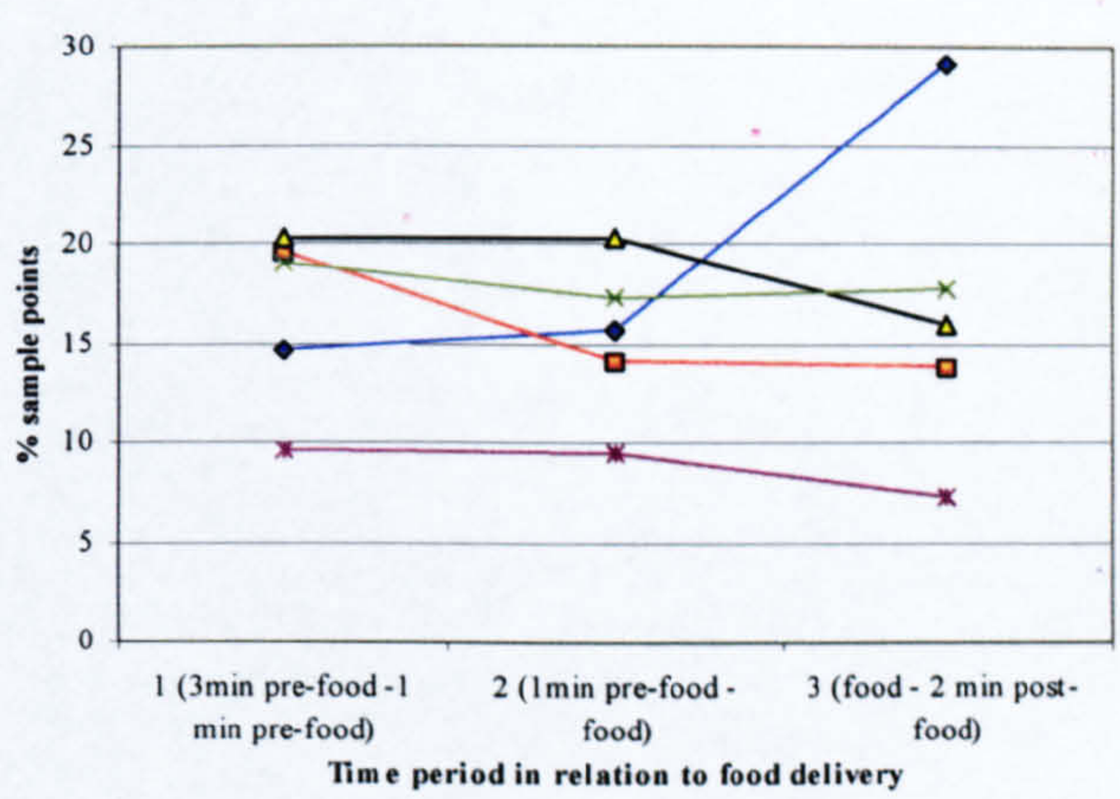
b. Trial period 2 (sessions 5-8)



c. Trial period 3 (sessions 9-12)



d. Trial period 4 (sessions 13-16)



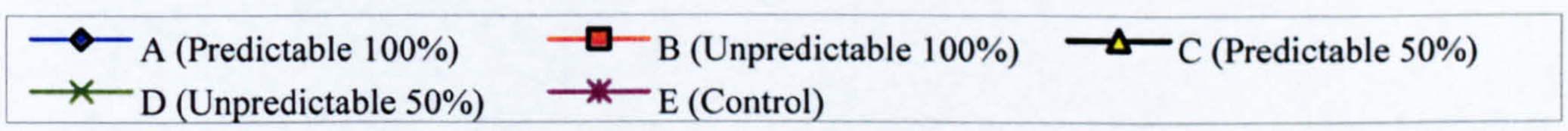
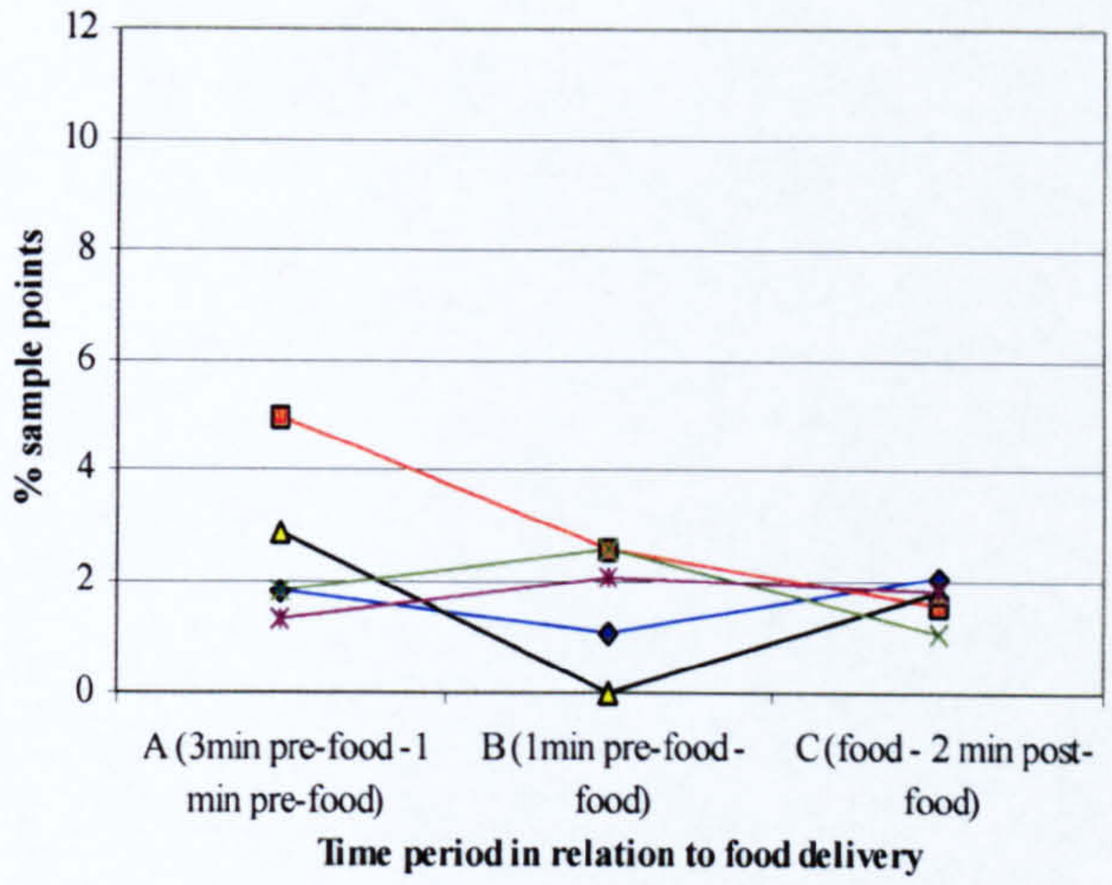
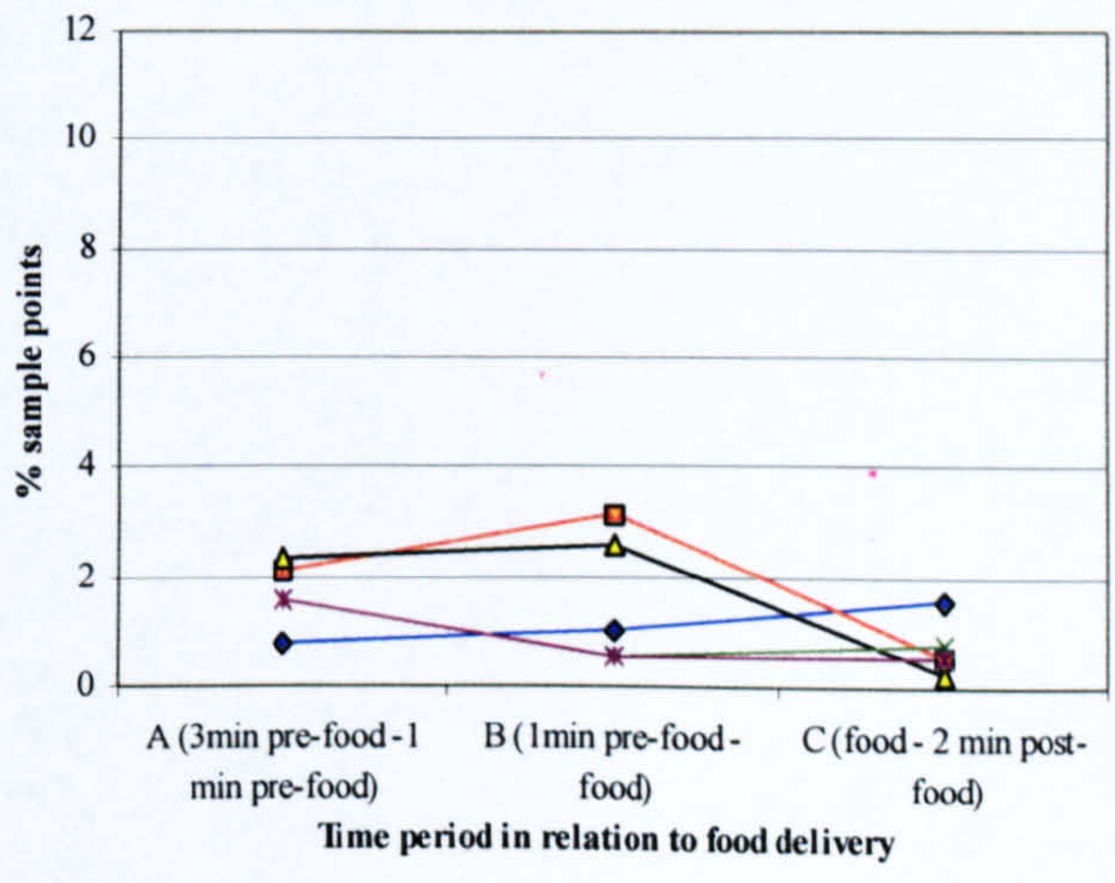


Figure 6.19 Interaction between 'Time Period' and 'Predictability of food delivery' for each of the four trial periods for 'Scent mark'

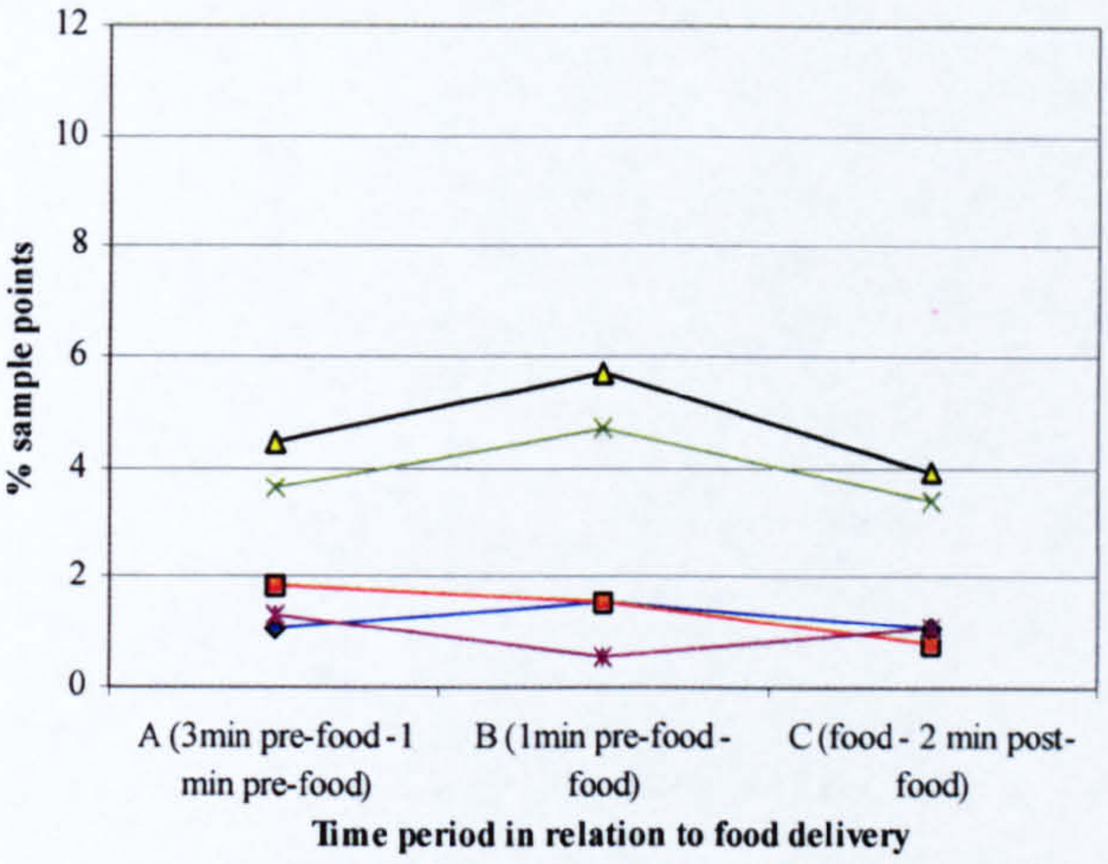
a. Trial period 1 (sessions 1-4)



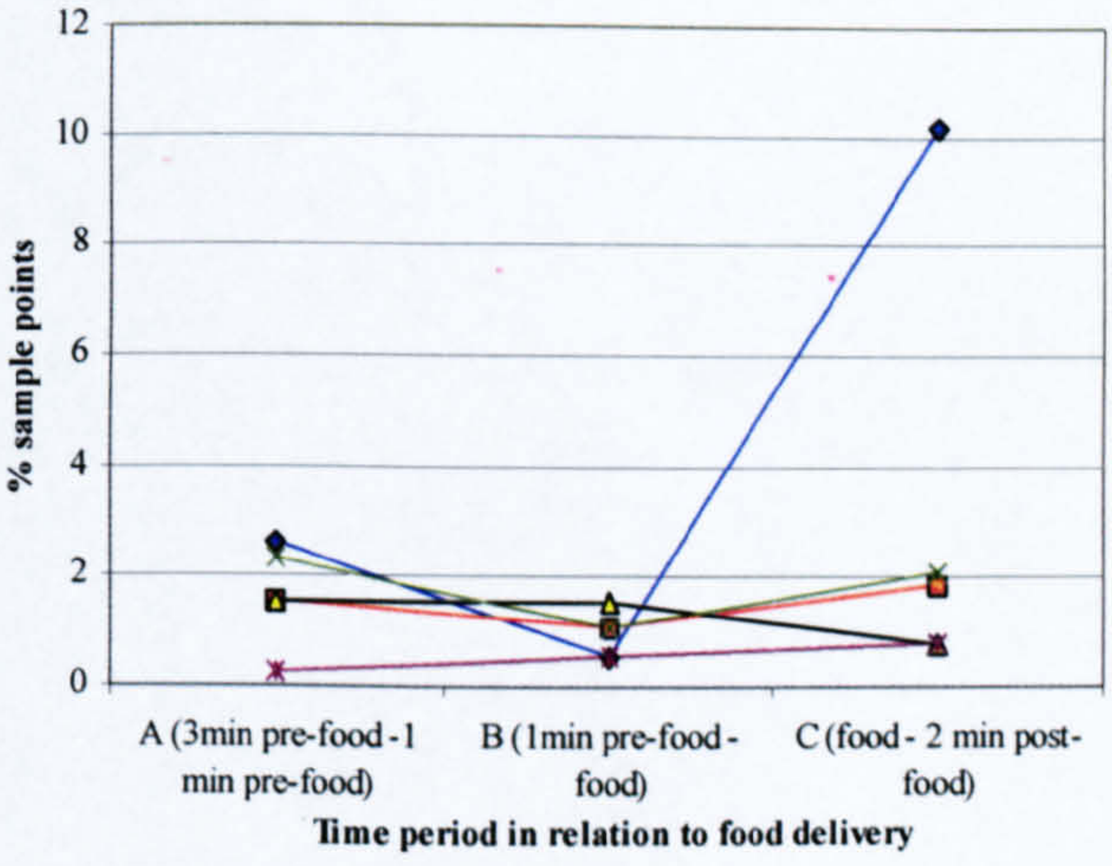
b. Trial period 2 (sessions 5-8)

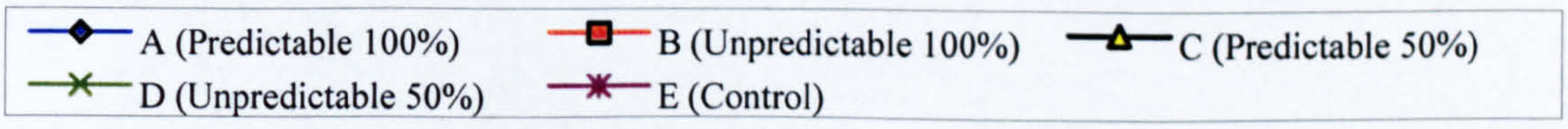


c. Trial period 3 (sessions 9-12)



d. Trial period 4 (sessions 13-16)





6.4 DISCUSSION

The results of this study indicate that manipulations in the time schedule and concurrent non-contingent reinforcement schedule of food delivery have important effects on the behaviour and welfare of the common marmoset. In the following discussion, each behavioural category will be considered in turn. The effects of predictability of feeding, trial period, time period and the various interactions of these variables will be discussed.

Inactive

All four experimental conditions showed a significant reduction in inactivity when compared to the Control condition. This is in accordance with the study described in Chapter 5, where the 'no signal' condition (equivalent to Condition B in the present study) also showed significantly lower rates of this behaviour when compared to the Control. A decrease in inactivity may be a sensitive measure of stress in the common marmoset (see Chapter 4). These results therefore initially suggest that receiving a desired piece of food from an observer may be a stressful experience for the animals. However, in the previous study, described in Chapter 5, there was no decrease in the amount of inactivity in comparison to the Control group in conditions where a signal was used. This suggests that the presence of a signal, whether or not it is reliable, helps to alleviate the stress associated with receiving food from the observer.

There was a significant main effect of trial period on this behaviour, with significantly higher rates in the second period compared with the first and third. It is difficult to explain these results. However, when the interaction between trial period and predictability of feeding was considered, it was evident that animals in the different experimental conditions reacted differently to the different stages of the study. Although there were significant main effects of both predictability of food

delivery and trial period, the results may be better explained in terms of the interaction between the variables.

For the first three trial periods (*i.e.* trials in which food was delivered), the rates of inactivity shown by Condition A animals (which received food at a predictable time on every trial) were virtually identical to those shown by Control animals. There was an increase between Trial Periods 1 and 2, possibly as the animals became accustomed to the experimental set-up and became less aroused simply by the presence of the experimenter. Rates were constant for Trial Periods 2 and 3 for both groups. However, whereas the Control animals showed constant rates for Trial Periods 2, 3 and 4, Condition A animals showed a marked decrease in inactivity at Trial Period 4. This apparent increase in stress was probably due to the absence of food, which they had received on a temporally predictable schedule on each of the previous 12 trials.

It appears, therefore, that receiving food on a temporally predictable schedule on 100% of relevant occasions causes little change in behaviour, or increase in stress, compared to animals not given any food. However, if food is not delivered when the animal has come to expect it, this may be very stressful, more so than for animals that had initially been receiving their food on a less predictable schedule. A reliable temporal schedule may therefore be good for welfare; deviations from the rigidity imposed by this may however override any welfare benefits so gained.

Condition B animals (*i.e.* those that received temporally unpredictable food on all trials) showed fairly constant levels of inactivity throughout the entire study period. This was also seen with the equivalent condition in the previous study. It is likely that the initial arousal caused by my presence was reduced as the animals became habituated to me, as was seen for Condition A and the Control condition. However, this reduction in arousal may have been cancelled out by increased

arousal due to the unpredictable feeding schedules, as the association between the schedule and the presentation of the reward was established.

Condition C animals (*i.e.* those that received food on a temporally predictable schedule on only 50% of trials) showed rates of inactivity in Trial Period 2 that were similar to those seen for Trial Period 1, as was the case for Condition B animals. However, there was a sharp decrease in rates of the behaviour for Condition C on Trial Period 3 that was not seen for Condition B. This suggests that stress levels continued to rise for those animals receiving their food on a temporally predictable schedule on 50% of relevant occasions, compared with on a temporally unpredictable schedule on 100% of occasions.

The pattern seen for Condition D animals (i.e. those that received food on a temporally unpredictable schedule on 50% of trials) was intermediate between that of Condition B and C animals. This is surprising, as Condition D animals received their food on the most unpredictable schedule and we might therefore have expected them to show the most stress-related behaviour if both types of predictability were affecting behaviour equally. However, it was the animals that received their food on the temporally predictable schedule only 50% of the time (Condition C) that displayed the least inactivity, which suggests that they were experiencing the highest levels of stress. These animals were subject to a moderately predictable schedule, which (along with Condition B, which was also moderately predictable) I had hypothesised would be the *most* beneficial in terms of welfare. These results suggest that receiving food on a temporally predictable schedule but not at every relevant opportunity is detrimental to welfare when compared with food delivered on an unpredictable temporal schedule, irrelevant of whether it is given on 100% or 50% of occasions. This suggests that receiving food on a temporally unpredictable schedule may buffer animals against delays, which appear to be detrimental if they occur on a temporally predictable schedule. The patterns seen for Conditions C and D in the present study are comparable to that for the 'unreliable signal' condition in

the previous study. This might indicate that an unreliable signal is similarly detrimental to welfare as these conditions. However, another factor that all three conditions have in common is that they all had a 50% rate of reinforcement. The effects seen may be due to this factor, rather than any differences in either signalled or temporal predictability.

Rates of inactivity for conditions B, C and D were similar for Trial Period 4 (the four sessions when food was not provided). It is interesting to note that this level was higher than that seen for Condition A animals. Again, it appears that receiving food on a schedule with some element of unpredictability may buffer animals against the harmful effects caused by unexpected changes to the feeding routine. Rates of inactivity remained most constant for animals that received food on an unpredictable schedule on 100% of initial trials (Condition B). This may be due to the fact that the schedule was moderately unpredictable, so afforded some protection against the detrimental effects of an unexpected missed or delayed feed, but was not so unpredictable that it caused a great increase in stress in itself.

There was a significant main effect of time period of observation on this behaviour also. Uncorrected values from post-hoc *t*-tests indicated that there was more inactivity in Time Period 2 than either Time Period 1 or 3. However, when the Bonferroni correction was applied to the post-hoc *t*-tests, these differences between the time periods became non-significant. Without being able to pinpoint significant differences between individual conditions, it is virtually impossible to draw any meaningful conclusions from these results. There was, however, a significant interaction between time period of observation and predictability of food delivery.

This interaction showed that the highest level of inactivity occurred for the Control animals. Rates of inactivity were fairly constant across the three time periods for all conditions except Condition A. Rates for Conditions B, C and D were similar, but lower than those seen for the Control condition. Rates shown by

Condition A animals were similar to those of the Control condition for the first two time periods, but dropped sharply in Time Period 3 (after the food had been delivered), to a level similar to that shown by the other experimental conditions. This decrease in inactivity could be due to the fact that food had just been delivered, and inactivity was reduced due to the foraging that occurred in response to this. This does not explain why a similar reduction in inactivity was not seen in the other experimental conditions, particularly for Condition B animals, which also received food on 100% of Trial Periods 1-3. However, inactivity was reduced to a rate similar to that of the other three experimental conditions in Time Period 3. It is possible that any differences in stress levels between the four experimental conditions were eliminated by the delivery of food.

There was a significant three-way interaction between predictability of feeding, trial period and time period. The most striking effect shown by this three-way interaction concerned Condition A animals, which received food at the same time on 100% of trials in the first three periods, but no trials in the fourth period. For the first three trial periods, rates of inactivity were similar to the Control condition for the first two time periods (before food was given) and decreased to a rate similar to that of the other three experimental conditions when food had been delivered. This suggests that receiving food on a predictable temporal schedule on all relevant occasions is less stressful than receiving it on a less predictable schedule in the time period before feeding, but makes no difference once food has actually been delivered. However, in the fourth trial period, rates of inactivity fell in comparison to the Control condition for Time Periods 1 and 2, becoming similar to those shown by the other three experimental conditions. This suggests that not receiving expected food, although associated with some increase in stress-related behaviour, may be no more stressful for animals accustomed to a highly predictable schedule than for those used to a less predictable routine. However, very low levels of inactivity - the lowest seen throughout the whole study - were seen for Condition

A animals in Time Period 3, after food would have been delivered in the initial stage of the study. It appears that animals accustomed to a highly predictable schedule of food delivery experience high levels of stress in the period directly following expected food delivery, if this food is not provided. This suggests, in common with findings of Shenger-Krestovnikova (described by Pavlov, 1927) and Levine and co-workers (1972) that loss of predictability of an appetitive event is more detrimental to welfare than a constant lack of predictability of a similar event.

The three-way interaction also indicated that differences between Conditions B, C and D were not evident until Trial Period 3, and had disappeared by Trial Period 4. This suggests that it takes longer for animals to become conditioned to a less predictable schedule than a predictable one. Receiving food on a predictable temporal schedule but not at every relevant opportunity appears to be more stressful than receiving it on an unpredictable temporal schedule, irrelevant of the proportion of trials in which food was provided. However, when food was not delivered according to the relevant schedule, there was no difference between these conditions in terms of this stress-related behaviour. Therefore, a moderately or highly unpredictable schedule may be equally beneficial in 'buffering' animals against the high levels of stress that seem to occur if feeding is unavoidably delayed or missed.

Locomotion

Increases in locomotion are thought to indicate increased stress in this species (see Chapter 4). A significant main effect of predictability of food delivery was seen for locomotion. Significantly more locomotion occurred in Conditions B, C and D than in the Control condition, whereas there was no significant difference between rates of locomotion in Condition A and the Control. These observations indicate that receiving food on a moderately or highly unpredictable schedule is more stressful than receiving no food. The fact that receiving food on a highly predictable schedule (Condition A) appeared to be no more stressful than receiving

no food indicates that it is not food delivery *per se* that was causing the increased locomotion and therefore, by analogy, stress. There was also significantly less locomotion in Condition A than Condition C, indicating that a highly predictable schedule (*i.e.* temporally predictable, with food delivered on all relevant occasions) is less stressful than a temporally predictable one in which food is delivered on only 50% of relevant occasions. Additionally, there was significantly more locomotion in Condition C than Condition B, although both of these conditions were moderately predictable. It seems that receiving food on a temporally unpredictable schedule on 100% of relevant occasions is less detrimental to welfare, as it causes less stress, than receiving food on a temporally predictable schedule on only 50% of occasions. It appears from these results that Condition C animals were experiencing the highest levels of stress, a conclusion that was also drawn from the effects on inactivity.

There was also a significant effect of trial period on locomotion, with significantly lower rates in Trial Period 2 than either Trial Periods 1 or 4. It is likely that habituation to the experimental situation was responsible for the lessened amount of locomotion observed in the second period compared with the first. The withholding of food on the last period was probably responsible for the increase in locomotion seen here. The effect of time period on locomotion showed that there was significantly less locomotion in Time Period 3 than Time Period 1. This is likely to be due to the delivery of food reducing the stress associated with feeding anticipation. However, conclusions based purely on interpretations of main effects may be over-simplified when significant interactions between the variables are also found.

A significant interaction was found between trial period and predictability of food delivery for locomotion. Rates of locomotion for Condition A animals were similar to those shown by the Control group, until Trial Period 4 when they rose sharply. This suggests that highly predictable schedules of food delivery are

beneficial to welfare in that they cause minimal stress when they remain highly predictable, but deviations from the routine cancel out any benefits as rates of stress rise sharply. However, rates of locomotion in Trial Period 4 were similar for Condition A as for Conditions B, C and D, suggesting that while stress did increase in this time period for Condition A, it was only to the same level as was associated with deviations from less predictable routines. Therefore, feeding on a highly predictable routine may still be desirable, as although stress may increase if delays occur, it is only moderate and would be over compensated for by the benefits seen when the routine is adhered to. This would be especially true if it could be ensured that delays were minimised as far as was practicable. This area clearly requires further study, as results from analyses of scratching and scent marking indicate that feeding on such a predictable routine could be quite detrimental to welfare, as very large increases in these stress-related behaviours were seen upon deviations to the routine.

Rates of locomotion for Conditions B and D were similar to each other, and constant throughout the four trial periods. They remained at an intermediate level, which was greater than was seen for the Control animals. This suggests that feeding on an unpredictable temporal schedule, regardless of whether food is given on all or only a percentage of relevant occasions, is associated with a moderate level of stress which has some buffering effect against the detrimental effects of a change to the routine. It could also be that animals used to an unpredictable feeding routine are less able to detect deviations from this routine and so are less affected by it than those exposed to a highly predictable routine. Animals in Condition C, one of the two moderately unpredictable conditions (fed on a temporally predictable schedule on 50% of trials) showed a peak in locomotion at Trial Period 3, the highest rate seen for any condition throughout the study period. This suggests that feeding on this type of schedule causes stress to increase during the study period, only decreasing when food is actually withheld in the final trial period.

This peak in locomotion was also seen for the most unpredictable condition in the previous study. However, it could also be due to the 50% reinforcement schedule that was used on both that condition and Condition C in the present study. The effect was not seen in Condition D in the present study, which also had a 50% reinforcement schedule. The most unpredictable condition, for which the peak in locomotion was seen in the previous study, was identical to Condition D in the present study, but with the addition of an unreliable signal. It is therefore possible that the unreliable signal, in association with an unpredictable temporal schedule, was responsible for the increase in locomotion, rather than the 50% reinforcement schedule.

The significant interaction between time period and predictability of feeding showed reductions in locomotion over the three time periods for Conditions B, C, D and the Control. The highest level of locomotion was shown by Condition C, and the lowest for the Control condition, as was the case for the interaction between trial period and predictability of feeding. Condition A animals showed similar rates to the Control condition for the first two time periods (before food was given) but rates increased at Time Period 3 (after food delivery). The significant interaction between trial period and time period showed, at Trial Periods 1 and 2, the highest rates of locomotion for Time Period 1, an intermediate amount at Time Period 2 and the lowest amount at Time Period 3. The least locomotion was seen in Time Period 3, after feeding which was to be expected. However, it is more surprising to find a reduction in locomotion in Time Period 2 compared with Time Period 1, as arousal might be expected to increase within anticipation of feeding, as the data collection period progressed, before the food was delivered. The reduction in locomotion in all three time periods at Trial Period 2 compared with Trial Period 1 is probably a result of habituation to the experimental situation. The increase in locomotion seen at Trial Period 3 compared with Trial Period 2 is possibly due to an increase in stress due to the animals learning the feeding schedule. It should be remembered

that all conditions are considered together for this interaction, so these conclusions may be over-simplified and will be elaborated on with consideration of the significant three-way interaction.

This three-way interaction between predictability of feeding, trial period and time period indicates that rates of locomotion were similar to Control Group values for Condition A animals for Trial periods 1-3. There was a sharp increase in rates of locomotion, resulting in a peak value, at Time Period 3 in Trial Period 4, when food was being withheld, suggesting a substantial increase in stress at this time. However, rates of locomotion were higher than Control values for Time Periods 1 and 2 in the final Trial period, which indicates a general increase in stress around feeding time. These results show that the increase in locomotion seen as a significant main effect in Trial Period 4 was due to this increase for Condition A animals only. It is also evident from the three-way interaction that the peak in locomotion seen for Condition A at Trial Period 4 at Time Period 3 was higher than for any other condition, except Condition C in Trial Period 3. It also emphasises the importance of consideration of significant interactions between variables when interpreting results such as these.

The increase in locomotion seen at Trial Period 3 appears to be due to the increase shown by Condition C animals. This concurs with patterns seen for inactivity, scent marking and vocalising, indicating that for this group stress increased throughout the study period, only decreasing when food was withheld. Therefore animals receiving food on a predictable schedule on 50% of relevant occasions may not experience stress as a result of the routine immediately, but stress levels may rise as time goes on.

Self-scratching

There was no significant effect of predictability of food delivery on rates of this behaviour. However, both trial period and time period had an effect on

frequency of this behaviour, and there was a significant interaction between predictability of feeding and trial period. There was more scratching seen in Trial Period 4 than Trial Period 1. There are two possible reasons for this. Firstly, stress levels may have increased throughout the study, only becoming significantly higher than initial levels by the fourth, and last, trial period. Secondly, the change in experimental procedure, that is the lack of food delivery, on the last trial period may have been responsible for increasing stress levels and causing a significant increase in scratching in the final trial period. The interaction between predictability of feeding and trial period showed that rates of scratching, and therefore also possibly of stress (see Chapter 4) remained low throughout the whole study period for Control animals and those in Condition B, which received food at an unpredictable time on every trial. Rates for Condition A animals were similar to these for the first three trial periods, but increased dramatically at Trial Period 4, when food was withheld. These results suggest that receiving food on either a highly or moderately predictable schedule, as long as it was given at some point on every relevant opportunity, caused minimal stress to these animals. However, the most predictable schedule could be detrimental to welfare in that deviations from it caused increases in stress. Conditions C and D both showed similar patterns, with rates of scratching increasing over the first three trials, reaching a peak at Trial Period 3, and decreasing at Trial Period 4. This suggests that not receiving food after being exposed to a schedule where food is given on only 50% of opportunities, is actually less stressful than continuing to receive it on this schedule. This is regardless of the temporal predictability of the schedule. The only difference between these two conditions was seen at Trial Period 3, indicating that any differences in stress caused by the different schedules took some time to develop. Changes to rates of scratching behaviour as a result of differing predictability of food delivery result in similar conclusions as were drawn based on observed changes in rates of inactivity, which increases confidence in the validity of these conclusions.

There was a significant effect of time period of observation on rates of scratching. However, post-hoc *t*-tests failed to show any significant differences between the time periods in rates of the behaviour after Bonferroni correction had been applied. Uncorrected values indicated that scratching was decreased after feeding compared to both pre-feeding periods. It is likely that stress levels were higher before feeding due to the anticipation of food delivery, and were decreased after food had been given. However, it is necessary to exercise caution when drawing this conclusion from these data, as significance was not reached in post-hoc tests when the appropriate correction had been made to guard against Type II errors.

Scent marking

Scent marking is thought to be an indicator of stress in this species (see Chapter 4), so it is likely that similar patterns of this behaviour should be observed as were seen for both locomotion and scratching. There was no significant main effect of predictability of feeding on scent marking, although there was an effect of trial period, and significant interactions between the factors. There was significantly more scent marking in the third trial period compared with the second. It is initially unclear why this should be the case, although when the interaction between predictability of feeding and trial period is considered, it seems that this difference is due to the increase in scent marking seen for both Conditions C and D in Trial Period 3. A similar pattern was seen as was found for locomotion and scratching (and also, inverted, for inactivity), and as changes in these behaviours are all thought to be indicators of stress in this species (see Chapter 4), this improves confidence in the conclusions drawn from those results. However, at Trial Period 1, there was more scent marking for Condition B than for any of the other four conditions. This may simply be an anomaly of the data, or it may be an indication that the temporally unpredictable schedule of giving food on every trial was initially fairly stressful, but became less so as trials progressed.

The interaction between time period and predictability of feeding indicates that at Time Periods 1 and 2, before food was given, more scent marking was seen in Conditions B, C and D than in Condition A or the Control condition. It appears that the more unpredictable conditions are associated with more stress than the most predictable condition. Similar findings were made in the previous study, where increased scent marking was found in the 'no signal' and 'unreliable signal' conditions. However, at Time Period 3, after food had been given, similar, fairly low, amounts of scent marking were seen for Conditions B, C, D and the Control condition, whereas much greater rates of the behaviour were seen for Condition A. This suggests that animals receiving food on a highly predictable schedule underwent less stress before feeding, but more stress after feeding, than those fed on a less predictable schedule. The significant interaction between time period and trial period shows that scent marking decreased at Time Periods 1 and 2 (*i.e.* the period before food would have been given) in Trial Period 4 (when food was withheld). However, it increased at Time Period 3 (*i.e.* the period after food would have been given) in the same trial period. Thus stress decreased after food was withheld in the time periods before it would have been given, but increased at the time periods after it would have been given. This is a similar pattern as was seen for locomotion, but in contrast to locomotion where rates only increased to an intermediate level, levels of scent marking rose to the highest level seen in the whole study.

However, the significant three-way interaction between the predictability of food delivery, trial period and time period indicates that the above discussion of the effects of the variables on scent marking behaviour is over-simplified. This interaction shows that the increased level of scent marking shown by animals in Condition A at Trial Period 1 only occurred for Time Period 1 (3-1 minutes before food delivery). This might have been due to a reduction in stress associated with habituation to the experimental situation and feeding schedule that occurred not only over sessions, but also within each individual session. However, habituation

within sessions was not seen in the initial study presented in Chapter 5. The increase in scent marking seen for both Conditions C and D in Trial Period 3 occurred over all three time periods. The increase in scent marking seen for Condition A was only seen at Time Period 3 in Trial Period 4, and reached a peak almost twice as great as for any other condition, trial or time period. This concurs with patterns seen for inactivity, locomotion and scratching. It shows that the stress experienced by animals experiencing a disruption to an otherwise highly predictable feeding routine occurs in the period after the expected food would normally have been delivered, rather than causing a more general increase in stress around the whole feeding period. It also suggests that the levels of stress associated with such a disruption are far higher than those associated with a more unpredictable feeding routine. Once again, it seems that loss of predictability is more stressful than lack of predictability. It appears from these data that the most beneficial routine for welfare is a temporally unpredictable one, when food is provided on a reinforcement ratio of 1. Such a routine appears to cause a certain amount of stress during its normal operation, but also to buffer animals against the detrimental effects of disruptions to it.

Rates of scent marking were higher for Conditions C and D at Time Period 2 in Trial Period 3. It is possible that any effects on scent marking seen as the study progressed, due to factors such as habituation, establishment of the routine or the change in the routine when food was withheld, might be more obvious in this period as it is directly before food delivery. The data collection period, albeit of only eight minutes duration, represents a microcosm of the day of a laboratory marmoset, or at least of the period around feeding time. The moment that food is delivered is likely to be the most salient point, as perceived by the animals, in the data collection period. Just as feeding is thought to be the most important event in the day of a captive animal (Carlstead, 1986) and therefore might be expected to have a major influence on behaviour (Waitt & Buchanan-Smith, 2001), feeding is likely to be the

most important point in the experimental period. Behavioural observations collected nearest in time to this point therefore might be expected to be most affected by feeding and the feeding schedule.

Scent marking was only significantly affected by stress in the study described in Chapter 4, when data from the two separate groups were considered together, resulting in a larger sample size. Increased scent marking may be a less sensitive measure of stress than either decreased inactivity or increased locomotion, and was therefore not so strongly affected by the relatively mild stressor employed in the earlier study. The fact that scent marking was so strikingly affected by deviations in predictability of feeding, particularly in respect to loss of predictability, suggests that the stress resulting from variations in predictability of feeding was fairly severe, at least in comparison to handling and removal of animals from their home cage and cage mate. This is despite the fact that the food whose predictability was manipulated was only a titbit, rather than the main meal of the day. This might have important implications for the results of this study, as it would mean that variations in predictability of feeding, and conceivably other husbandry events, have the potential to cause severe stress to captive animals.

Foraging

Compared with their effects on other behaviours, the experimental variables had straightforward effects on foraging behaviour with no interactions being found. Differences in amounts of foraging seen as a result of predictability of food delivery can be explained simply by the amount of food available. The Conditions A and B, where food was given on 100% of the first three trial periods, showed significantly more foraging (around twice as much) as Conditions C and D, where food was only provided on 50% of these occasions. Control animals, which were never provided with food, showed significantly less foraging than any of the experimental conditions.

Unsurprisingly, there was significantly more foraging in Trial Periods 1, 2 and 3 than Trial Period 4, as food was provided on the first three periods but not the fourth. Similarly, significantly more foraging was seen at Time Period 3 (after food was delivered) compared to Time Periods 1 and 2 (both before food was delivered).

6.5 SUMMARY AND CONCLUSIONS

The results of this study indicate that differences in temporal predictability and ratios of reinforcement rates, in relation to feeding, may have a profound influence on the behaviour and welfare of captive animals. Stress levels in the common marmoset were determined from changes in rates of inactivity, self-scratching, scent marking and locomotion, all of which are thought to be indicators of stress in this species. Feeding on a predictable temporal schedule and on 100% of trials (overall, a highly predictable schedule) resulted in stress levels virtually unchanged from those of Control animals, which received no food and were subject to no other interaction from the experimenter. Providing food on all trials, but on an unpredictable temporal schedule (overall, a moderately predictable schedule) resulted in stress levels that were slightly higher than those seen for the highly predictable schedule. Food delivered on a predictable temporal schedule but on only 50% of trials (again a moderately predictable schedule) was associated with the highest levels of stress. A delivery rate of 50% concurrent with an unpredictable temporal schedule (the schedule with the lowest overall predictability) resulted in stress levels intermediate between the previous two.

It would appear from these results that using a highly predictable schedule, where food is delivered at the same time each day should be the most beneficial in terms of welfare. Additionally, no feeding related cues should occur when feeding is not about to happen (*i.e.* feeding should occur on 100% of relevant occasions). However, when the schedules were disrupted by not providing food on four

consecutive trials, changes in the above behaviours indicated a sharp rise in stress levels for animals fed on the highly predictable schedule. This did not occur for the three less predictable schedules, and resulted in stress levels higher than those seen for these groups at any point in the study period. Therefore, although feeding on a highly predictable schedule may be most beneficial when the routine is rigidly adhered to, any deviations from this may be extremely detrimental to welfare. In comparison, the less predictable schedules appeared to offer some protection against the detrimental effects of disruptions to the routine, possibly as animals were less able to detect deviations from a these schedules.

It is unrealistic to expect all feeding delays, and hence disruptions to schedules, to be eliminated in a real-life situation. Additionally, it is unlikely that all non-relevant feeding-related cues could be eliminated, especially where many groups of animals are housed and there are therefore many feeding-related stimuli. Of the conditions used in the study in which food was delivered on 50% of trials, the temporally unpredictable schedule tended to result in lower levels of stress-related behaviours than the temporally predictable one. Therefore, a temporally unpredictable feeding routine appears to be the most appropriate if welfare is to be maximised. This would result in a moderate level of stress around feeding time, which would not be increased if deviations from the routine were unavoidable.

The previous study (described in Chapter 5) indicated that the inclusion of a reliable signal, before a temporally unpredictable food delivery, might help to reduce the stress associated with such a schedule. It would appear from the results of the two studies that feeding animals on a temporally unpredictable routine, but preceded by a unique and reliable signal, may optimise their welfare. This would result in a routine that is fairly low in stress, as seen in Chapter 5, but which enables animals to better cope with delays to feeding, as seen in the present study.

Although it appears that a temporally unpredictable routine may protect against the effects of feeding delays, it is unfortunate that the study on reliability of

signals (Chapter 5) did not specifically look at the potential buffering effects of signals in the case of delays to feeding. For example, it would be extremely useful to know whether animals conditioned to hearing a signal before feeding would suffer less from feeding delays if their signal was not heard, in comparison with animals that never received such a signal. It would also be useful to know whether stress would ensue if animals heard their signal, but feeding did then not follow it. This latter point is specifically addressed in the following study, which also attempts to put the highly experimental work of this and the previous chapter into a more realistic setting.

Chapter 7

Effects of reliability of feeding-related signals on the behaviour and welfare of stump-tailed macaques *(Macaca arctoides)* in a real-life setting

7.1 INTRODUCTION

7.1.1 The stump-tailed macaque

The stump-tailed macaque *(Macaca arctoides)* (I. Geoffroy, 1831) is a relatively large, powerfully built macaque with a short tail, after which it is named (Bertrand, 1969; Fooden, 1989). The Latin name of this species is derived from the Greek *arktos,* - 'a bear' and *eidos,* - 'apparent shape or form'; 'bear-like' (Gotch, 1995). Wild females weigh approximately 8.4kg, while males are heavier at around 12.2kg (Fooden, 1989), although captive specimens often weigh more (Chamove, 1981; Trollope & Burton-Jones, 1970). The long, shaggy pelage is reddish brown in adults and whitish in the neonate, while the skin of the face is bright pink or red and darkens with age and exposure to sunlight (Groves, 2001).

Stump-tailed macaques are found in dense broadleaf-evergreen forests of southern China and Southeast Asia (Fooden, 1989; Roonwal & Mohnot, 1977) and are classified as vulnerable by the IUCN (IUCN, 2002). They are largely terrestrial, foraging and travelling on the forest floor, but occasionally also foraging in trees, and usually sleeping in tall trees (Bertrand, 1969; Fooden *et al*, 1985). They may also flee into the canopy when alarmed, although it is more usual for them to flee on the ground (Bertrand, 1969). Stump-tailed macaques feed on fruit, seeds, young leaves and animal prey such as insects, birds and eggs (Richard *et al*, 1989). In the wild, this diurnal species wakes at around dawn, feeding until around 1000-1100h, and rests and grooms until around 1700h, when it feeds again before retiring at around dusk (Bertrand, 1969; Fooden *et al*, 1985). Natural group size ranges from

two or three to around 60 individuals, with a median group size of around 25 (Fooden, 1989). Groups are multimale-multifemale, with a ratio of males to females of around 1:6 (Fooden, 1989). Captive studies have shown stump-tailed macaques to observe a strict dominance hierarchy (Bernstein, 1980).

The tractable nature of the stump-tailed macaque led to it being recommended as a laboratory animal in preference to the rhesus macaque *(Macaca mulatta)*, which were described as being of a 'belligerent disposition' (Orbach & Kling, 1964; p. 343) (Kling & Orbach, 1963b; Orbach & Kling, 1964). The stump-tailed macaque, by contrast, was said to be docile towards humans and easily manageable (Orbach & Kling, 1964). In 1969, Bertrand stated that this species was beginning to be extensively used in biomedical research. However, perhaps because of its relatively low gross reproductive rate (Fooden, 1985) and tendency to become obese in captivity (K. Morris, pers. comm.), it is currently not a widely used laboratory primate. Only one laboratory in the United Kingdom, the MRC Reproductive Biology Unit in Edinburgh, currently uses this species, and this institution also houses the UK's only breeding group (K. Morris, pers. comm.).

7.1.2 Measures of welfare in the stump-tailed macaque

The performance of displacement activities may provide insight into an animal's emotional state (Maestripieri *et al*, 1992) and, by inference, its welfare. Bertrand (1969) describes self-scratching as a displacement activity in stump-tailed macaques. He found that this behaviour increased in tense situations, such as when the animal was stared at by the observer. Bertrand (1969) also considers yawning to be a displacement activity in this species. 'Tension yawning' is distinguished from normal yawning in that 'it is repeated and the teeth are displayed' (Bertrand, 1969, p.95). Increased scratching and yawning were seen in this species in response to electrical and pharmacological activation of the locus coeruleus, a noradrenic nucleus in the brain that is considered to be implicated in anxiety (Redmond &

Huang, 1979). This physiological and pharmacological study increases the validity of these behaviours as welfare indicators.

Feeding may also occur in the stump-tailed macaque, out of context, as a displacement activity. Bertrand (1969) found that displacement feeding was sometimes observed when a macaque was 'frightened or uneasy' such as when approached by a more dominant animal (Bertrand, 1969, p. 44). In such situations, feeding began abruptly, and the macaques were observed to chew and sometimes swallow items that they would not normally eat, such as 'leaves of Virginia creepers, bits of wood or bark' (Bertrand, 1969, p. 45).

Stump-tailed macaque victims of aggression, but not aggressors, show increased autogrooming following aggressive conflict (Call *et al*, 2001) as well as in 'situations involving tension or conflict between two alternatives' (Bertrand, 1969, p. 175). Enclosing a stump-tailed macaque in a small space resulted in increased rates of autogrooming (Goosen, 1974b). Autogrooming was increased when the macaques were prevented from allogrooming (Goosen, 1974a). Bertrand (1969) also found that stump-tailed macaques exhibit increased allogrooming during situations relating to excitement or tension (Bertrand, 1969). They show increased agonistic and mounting behaviour during periods of social tension, such as following the introduction of previously separated group members (Bernstein *et al*, 1992; Bertrand, 1969; Slob & Nieuwenhuijsen, 1980). Rates of vocalisation, consisting almost entirely of high-pitched screams, peaked in stump-tailed macaques prior to feeding in conjunction with increases in self-directed, abnormal and aggressive behaviours (Waitt & Buchanan-Smith, 2001). Increases in vocalisation were therefore interpreted in this study as indicators of stress in these macaques. Similarly, abnormal behaviours such as eye poking, pacing, wall-licking, self-clasping and rocking were interpreted as being indicative of reduced welfare in the same study.

Stump-tailed macaques may show self-aggression (SA), often as a consequence of experiencing conditions of social deprivation during rearing (Anderson & Chamove, 1981). SA in this species generally consists of the animal directing vocal and visual threats towards its own limbs, often also self-slapping and -biting (de Monte *et al*, 1992). Potentially frustrating situations, such as watching a companion receiving food treats from a caretaker, resulted in increases in SA in stump-tailed macaques prone to these behaviours (de Monte *et al*, 1992). SA, as well as being a possible indicator of anxiety, may also be detrimental to welfare in itself as serious injury may result from self-aggressive behaviours (Anderson & Chamove, 1981). For the purposes of the thesis, SA is included in the 'abnormal behaviour' category.

In conclusion, increases in scratching, yawning, feeding, autogrooming, allogrooming, agonism, mounting, vocalising and abnormal behaviour may be indicative of anxiety and reduced welfare in this species. Increases in inactivity may also be undesirable, especially as captive stump-tailed macaques, and in particular this study population, are prone to obesity (K. Morris, pers. comm.). However, the fact that these behaviours comprise all but one of those defined in Table 3.6 emphasises the need for caution in interpretation of the results. Significant increases in the rates of any behaviour (except for the remaining category, locomotion) might be interpreted as being indicative of reduced welfare. This is nonsensical, as we might expect *improved* welfare to result in behavioural changes, a possibility that is scarcely accommodated by the described behaviours.

Interactions between the variables might also be difficult to interpret; for example, should we see increased scratching but decreased autogrooming it would be tenuous to conclude that welfare was compromised. In common with data discussed in Chapter 4, a cautious and pragmatic approach is necessary to avoid misinterpretation.

7.1.3 Aim of the present study

The present study examined the effects of food-related signals on the behaviour of stump-tailed macaques. It was intended to test further the hypotheses presented in Chapter 5; that is, that unreliable signals preceding food delivery would have a negative effect on welfare, whereas reliable signals would be relatively beneficial to welfare. One of the main drawbacks of the study described in Chapter 5 was its artificiality and I wanted to assess whether similar results would be obtained in a more natural situation. The present study therefore tested the same concepts as the earlier one, but in a more applied setting, and was designed to be complementary to it. Similar findings in the present study would add validity to the results presented in the Chapter 5 and further support the ideas discussed therein. Additionally, before extrapolating the implications of the previous study in order to potentially improve the welfare of captive animals, it was vital to assess whether the results could be replicated in a real-life setting.

It was hypothesised that similar results would be found as were seen in Chapter 5. In Chapter 5, three experimental conditions were used: 'reliable signal'; 'no signal' and 'unreliable signal'. The 'unreliable signal' condition was associated with the highest rates of stress-related behaviours, and it was expected that this effect would also be seen in the present study. Here, four experimental conditions were used: 'unreliable signal'; 'no signal'; 'reliable signal' and 'false signal'. As the 'false signal' condition used a signal that is essentially unreliable, it was expected that more stress-related behaviours would be seen in both the 'unreliable signal' and 'false signal' conditions. Few significant differences were found in behaviour between the 'reliable signal' and 'no signal' conditions in the previous study; it was expected that similarly few differences would be seen here.

7.2 METHODS

7.2.1 Study animals and housing

The study animals were nineteen adult stump-tailed macaques, housed in five separate groups at the Medical Research Council Reproductive Biology Unit, Bush Estate, Edinburgh. Groups ranged in size from three to five individuals, with two groups all-female, while the other three each contained one male. See Chapter 3 (page 77) for details of group compositions. A sixth group of animals, consisting of a male, several breeding females and their offspring, was not included in the study as it was felt that differences in group size and composition might affect the results. See Chapter 3 for details of housing and husbandry.

7.2.2 Feeding routines

Animals were generally fed once a day, although on some occasions 'extras', such as yoghurts, were given at other times. On weekdays, the mean time for the first group to be fed this main feed was 1239h (*SD* ± 8 minutes, n=62); the last group was fed at a mean of 1310h (*SD* ± 6 minutes, n=62). There was a range of between 13 minutes and 91 minutes, with a mean of 31 minutes (*SD* ± 14 minutes, n=62) between feeding the first and the last groups.

Generally, feeding began with one of three technicians entering the food preparation area and making up two buckets of food. The food consisted of a mixture of fresh chopped fruit and vegetables, such as apples, pears, oranges, bananas, cabbage, cucumber and carrots. A scoop full of dry food, such as primate chow, raisins and dried dates, was added to the bucket. The technician then walked down the corridor, opened the door to one of the cage rooms, entered each of the adjoining animal rooms in turn and scattered the contents of a bucket of food on to the shelves and among the wood shavings. The technician then returned to the food preparation area, and the process was repeated until all six groups of animals had been fed (see Plate 14 and Figure 7.1).

Plate 14 Technicians' corridor – entrances to macaque 'cage' and 'gang rooms' seen on left of picture, food preparation area on right

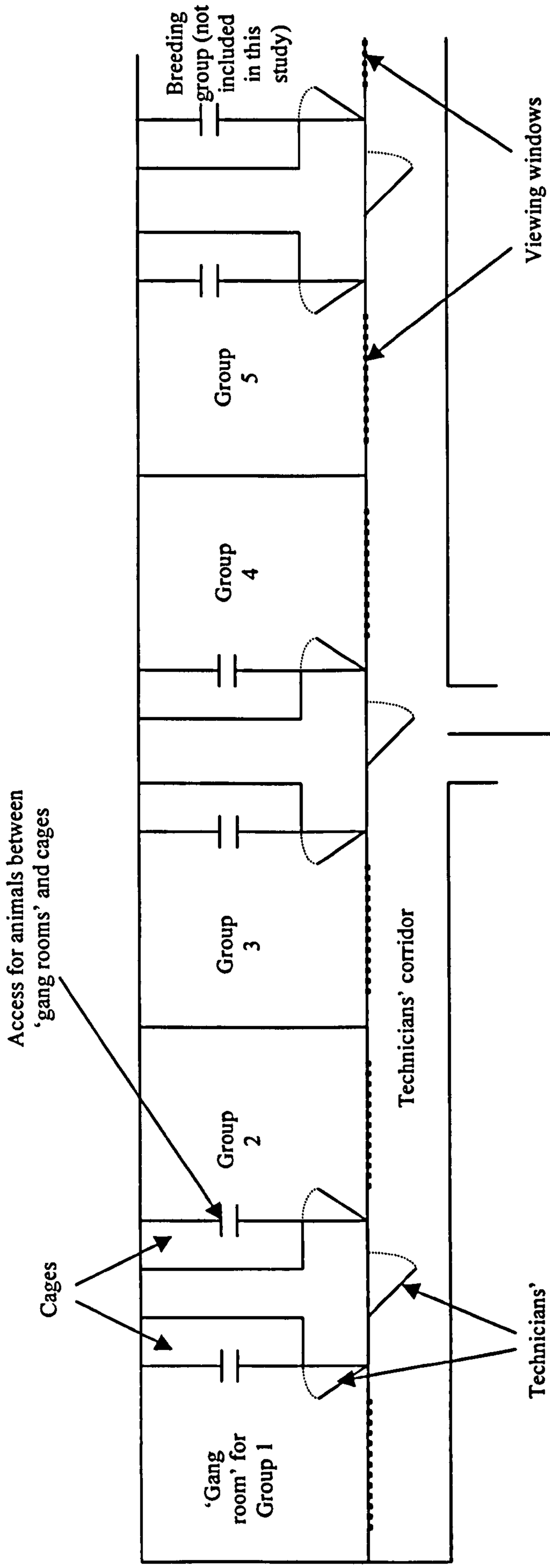


Figure 7.1 Plan of macaque housing at the MRC Unit, Edinburgh (not to scale)

The food preparation was generally accompanied by noises that the animals might associate with feeding, such as technicians moving around the building, keys jangling or food being chopped. One particularly salient noise was that of the dry food being emptied into the buckets. The loudness of this depended on the order that the food was put into the bucket; if it was put in first, it generally made more noise than if it was put in on top of other food. Each technician had their own method of making up the food, so the loudness and order of these feeding-related signals would depend on who was doing the feeding each day.

7.2.3 Experimental design

A repeated-measures design was used to investigate the effects of signal reliability on the behaviour of the macaques. Each group was therefore exposed to all four conditions. It was not possible to balance the order in which the conditions were presented to the groups; signal conditions were presented in the order A-B-C-D to all groups. The experimental procedure and behavioural observations were conducted on weekdays only, as it was not possible to gain access to the laboratory at weekends. The laboratory routine was quite different at weekends, when fewer technicians were on duty. No cleaning took place and animals were fed much earlier, at around 1000h. Conditions were as follows:

Condition A – Unreliable signal

In this condition there was no manipulation of existing routines. Animals were sometimes exposed to feeding-related noises, the most salient of which appeared to be the sound of the bucket being filled. This tended to occur at around 2-3 minutes before food was delivered to the first group, although the time lag between the two events could be as long as 90 minutes. The delays that sometimes occurred between these feeding-related noises and actual presentation of the food rendered them unreliable. Therefore signalled predictability of feeding was low. Groups were fed in a random order, although Groups 1 and 2 were fed within 1

minute of each other, as were Groups 3 and 4. Four data collection periods took place, for each group. This stage of the study lasted for a period of six weeks.

Condition B – No signal

In order to investigate whether the presence of unreliable signals had an effect on behaviour, in the second condition they were, as far as was practicable, removed. Food buckets were made up at around 0900h, as quietly as possible and with a radio playing to try and mask any sound that might occur. This radio was usually played all day and therefore in itself was not a cue that the food was being prepared. At the normal feeding time (*i.e.* at around 1230-1300h), technicians fed the groups as quietly as possible. Unfortunately certain feeding related signals, such as doors to animal rooms being opened, could not be eliminated, and the further through the feeding process the technician progressed, the more of these signals would have inevitably occurred. I therefore always watched one of the two groups that would be fed first. In order that my presence outside the viewing window did not itself become a reliable cue to feeding, I spent other periods watching the animals when food was not delivered. I could potentially have been an unreliable cue to feeding, but this was unavoidable without the use of a video camera. Groups were fed in a random order. This stage of the study again took six weeks, with four sets of observations collected for each group.

Condition C – Reliable signal

A unique reliable signal was introduced. Three different signals were used. A horn was sounded two minutes before Group 1 was fed. Group 2 was always fed immediately following Group 1, three minutes after the horn was sounded. A bicycle bell was rung two minutes before Group 3 was fed. Group 4 was fed immediately following Group 3, three minutes after the bell had been rung. Groups 1 and 2 therefore had the same signal, as did Groups 3 and 4. For Group 5, a whistle was blown two minutes before the feed was given. See Table 7.2 for a summary of signals and timings used for each group.

Table 7.2 Summary of signals and timings used for each group in Condition C

Group	Signal	Time between sounding of reliable signal and food delivery
1	Horn	2 minutes
2	Horn	3 minutes
3	Bicycle bell	2 minutes
4	Bicycle bell	3 minutes
5	Whistle	2 minutes

Each signal was presented once each weekday, followed by feeding after the appropriate interval. The connection between signal and food was therefore reinforced daily for each group, whether or not behavioural observations were being made of them. In this condition, feeding order was again random, but with Group 2 always following Group 1 and Group 4 following Group 3. Observations commenced as soon as the signals were introduced; the animals were not given a period of time in which to make the association between feeding and the signal. Four observation sessions were again collected per group in this condition, over a period of six weeks.

Condition D – False signal

In the final condition, the signals were sounded but were not followed by feeding for the group that was being observed, as they had been in Condition C. For example, if Group 1 were being observed, the horn was sounded, but neither Groups 1 or 2 received their food. After a temporally unpredictable period (mean = 17.3 minutes, SD ± 2 minutes, n=10), the horn was sounded again, followed by feeding two minutes later for Group 1 and three minutes later for Group 2. The first presentation of the signal therefore was not followed by the food; the signal was 'false'. At the second presentation, however, the signal was once again a reliable indicator of food delivery. Two observations were made of each group being presented with the false signal. The fact that the same signal was used for two groups in the case of the horn and bicycle bell, and that feeding times for the groups

sharing the signals were consequently related, meant that Groups 1-4 each received a false signal four times, but Group 5 just twice.

In order to reinforce the relationship between the signal and food delivery, reliable signals continued to be presented throughout the period of Condition D, with each group only receiving one false signal per week. Data collection for this condition took place over a period of four weeks. See Table 7.3 for a summary of experimental conditions.

Table 7.3 Summary of experimental conditions used in the study, and signals present in each condition

Condition	Signals present		
	Naturally occurring food-related sounds	Novel reliable 'signal'	False signal
A (Unreliable signal)	✓	×	×
B (No signal)	×	×	×
C (Reliable signal)	×	✓	×
D (False signal)	×	✓	✓

7.2.4 Data collection

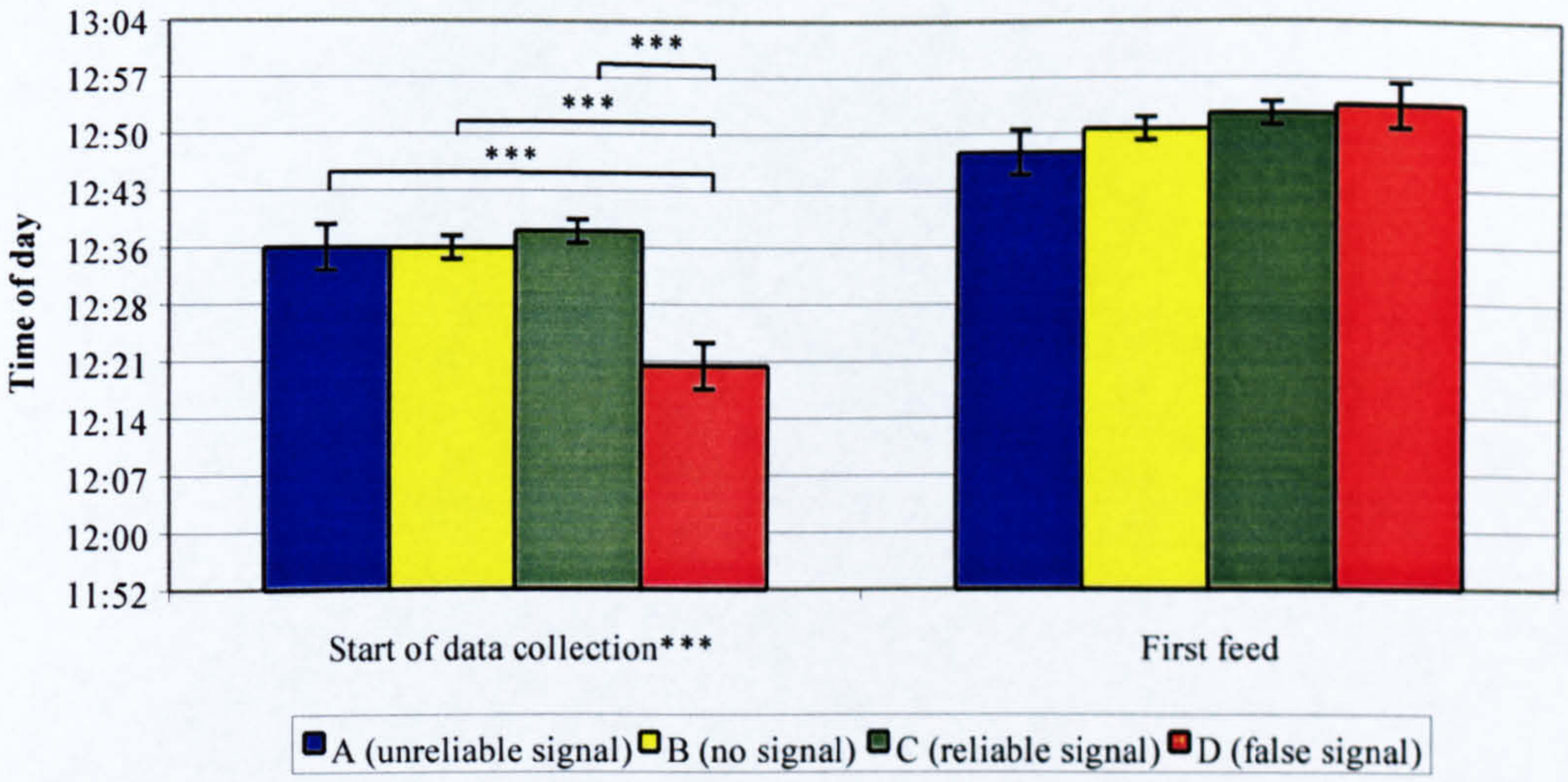
Data were collected on a palm-top computer, using THE OBSERVER 3.0 software (Noldus, 1993). Instantaneous scan sampling was used, with an interval of 30 seconds between scans. Behaviours recorded included: abnormal, affiliative, agonistic, autogroom, foraging, inactive alert, locomotion, self-scratching and vocalising (see Chapter 3, page 90, for behavioural definitions).

Although the time of the first feed did not significantly differ between the four conditions ($F_{3,56}$=1.50; p=0.22; n.s.), a one-way ANOVA showed that significant differences existed between conditions on the time that data collection began ($F_{3,56}$=9.31; p<0.001). Post-hoc Tukey tests showed that the time of the start of data collection for the false signal condition was significantly earlier than those for the other three conditions (see Table 7.4 and Figure 7.3).

Table 7.4 Results of post-hoc Tukey tests for effects of condition on time of start of data collection

Comparison between conditions	Mean Difference	S.E.	p
A-B	0:00	0:02	0.99
A-C	0:02	0:02	0.88
A-D	0:14	0:03	<0.001***
B-C	0:01	0:02	0.99
B-D	0:03	0:03	<0.001***
C-D	0:16	0:03	<0.001***

Figure 7.3 Mean times of start of data collection and delivery of first feed for each condition (bars represent Standard Errors)



*** p<0.001

Asterisks by x axis label indicate significance of ANOVAs. Asterisks above boxes indicate significance of post-hoc Tukey tests.

7.2.5 Statistical analyses

Observational data from 12 minutes prior to the signal until the signal (Period 1) were analysed, as were data from the 2 minutes immediately following the signal (Period 2). Data from the period that the technician was actually present in the room with the animals were not included in the analysis, as differing relationships between the different personnel and the animals may have had a

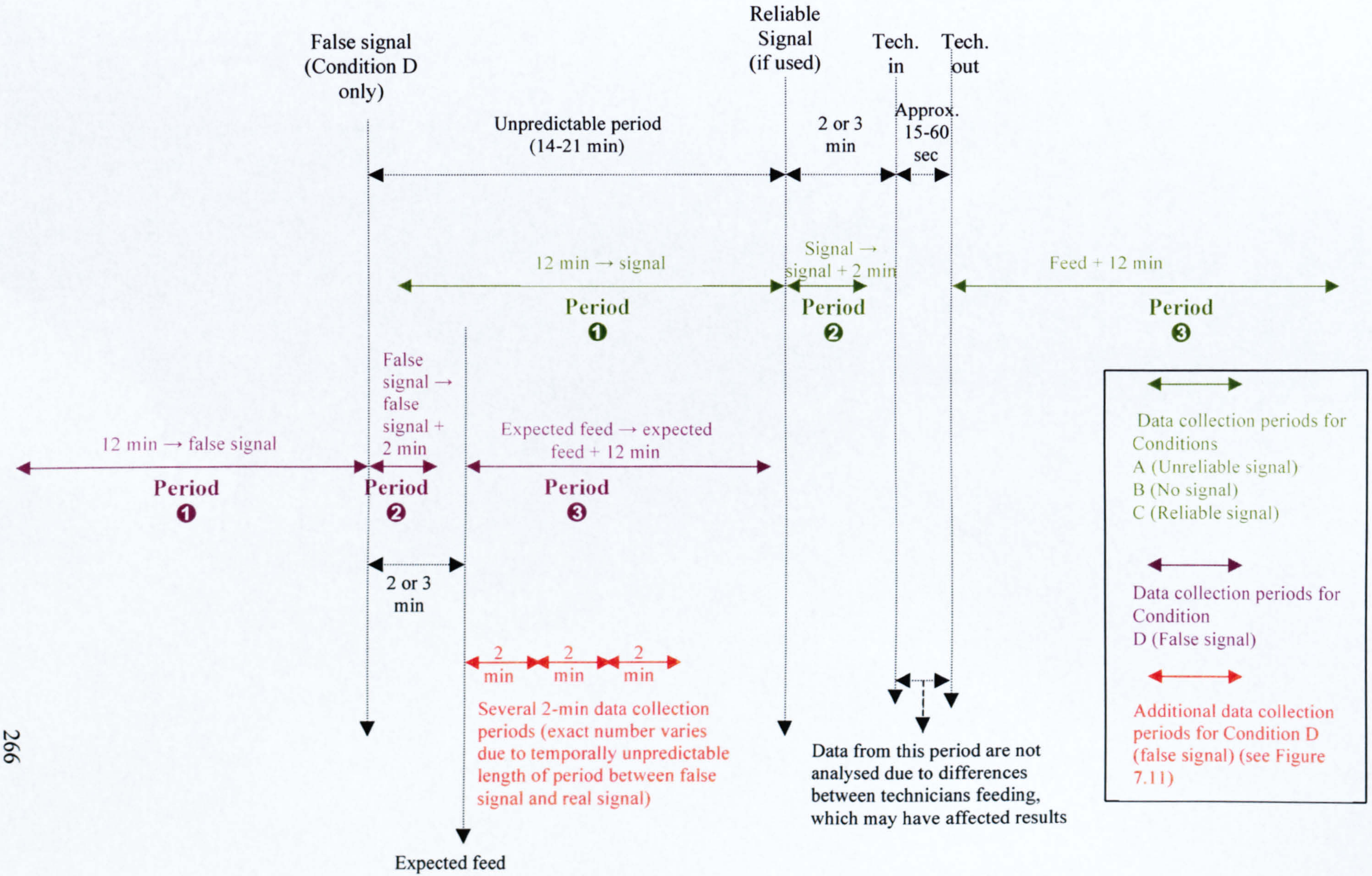
bearing on the animals' behaviour. However, data from the 12 minutes immediately following the technician's departure from the room were included in the analysis (Period 3) (see Figure 7.2 for representation of time periods used).

Data for Condition D (false signal) comprised the 12-minute period prior to the false signal (Period 1) and the 2-minute period immediately following the false signal (Period 2). Data for Period 3 were intended to correspond directly to those for Period 3 in Conditions A, B and C, yet had to relate to the false signal rather than the reliable signal that was given subsequently. These data were therefore taken from either 2 or 3 minutes after the false signal, according to whether the respective group had received food 2 or 3 minutes following the reliable signal, and so coincided with the time that the expected feed would have been delivered had the signal been reliable. A 12-minute period was used for this Period 3, in common with Conditions A, B and C (see Figure 7.2).

The behaviour of each animal was likely to have been affected by others in its group, and therefore could not be considered to be independent. For this reason, means were taken for each group, producing mean percentage activity budgets for the whole group. It was also possible for animals to go 'out of view' for some or all of the observational period. Rather than calculating an activity budget for each animal, based on their time in view, and then taking a mean of these, it was decided to add together all sample points for each behaviour and then calculate overall activity budgets for each group based on the sum of these data.

This method had the advantage that each behavioural observation, rather than each individual, had equal weighting in the analysis. It was felt that the converse, in which each individual animal had equal weighting, irrelevant of the amount of time it spent in view, might be less representative of the behaviour of the group as a whole. For example, if an individual was only in view for one or two scan samples, a very small proportion of the observational period, and by chance was behaving in an unusual manner at these sample points, this behaviour would be

Figure 7.2 Timing periods used in the study



over represented in the final analysis as it would appear that the individual spent the whole session performing it. Additionally, the behaviour of less frequently observed individuals may be less representative of their whole behavioural repertoire as it is possible that they might only perform certain behaviours when out of sight. For this reason, despite the fact that it meant that the behaviour of certain individuals might be under represented in the final mean activity budget, each behavioural data point was given equal weighting in the final activity budget and subsequent analysis. The three separate periods (*i.e.* 12 minutes pre-signal, 2 minutes post-signal and 12 minutes post-feeding) were of unequal length, so mean percentage sample points spent in each behaviour were calculated for each group.

Four sets of observational data were collected for each group in Conditions A-C, whereas two were collected for Condition D. For each condition, an overall mean activity budget was calculated from these repetitions. Therefore, one behavioural time budget was obtained per group for each of the three observation periods (pre-signal, post-signal but pre-feeding, and post-feeding) in each condition (A-D). See Table 7.5 for a summary of variables that were used in the analysis.

Table 7.5 Variables in the study, and number of levels within each

Variable	Within/between subjects	No. of levels	Levels
Presence / reliability of signal	Within-subjects	4	Condition A – Unreliable signal Condition B – No signal Condition C – Reliable signal Condition D – False signal
Time period of observation	Within-subjects	3	Period 1 – Pre-signal[1] Period 2 – Post-signal, pre-feeding[1,2] Period 3 – Post-feeding[2]

[1] Where applicable – signals were not given in Condition B
[2] Where applicable – food was not given in Condition D

Data were found to be normally distributed and so parametric tests were used. The following analyses were carried out.

Effects of 'Signal Condition' and 'Time Period' of observation

A two factor repeated measures analysis of variance (ANOVA) was used to determine overall behavioural differences associated with the study variables. Significance was set at alpha <0.05. Where significant differences were found, post-hoc multiple *t*-tests were used to pinpoint where significant differences lay. Use of *t*-tests for post-hoc comparisons, however, may increase the risk of Type 1 errors - finding significant differences between means where such a difference does not actually exist. The chances of making these errors increase with the number of levels of the independent variable (Everitt, 1996). In order to combat this problem, the Bonferonni correction may be used. This involves dividing the appropriate significance level by the number of *t*-tests performed. Therefore, for a significant difference to be assumed, the *t*-test must show significance beyond the 0.05 level. If a large number of *t*-tests are performed, however, this method may be highly conservative and Type II errors may be made, with some 'significant' differences missed (Everitt, 1996). For this reason, in the following results section, *t*-test results are reported both with and without the Bonferonni correction. Interactions between the variables were also analysed using the ANOVA.

Effects of time after the false signal in the absence of the expected food

To assess whether any behaviours increased or decreased with time after the expected feed, the time period between the false signal and the subsequent real signal (Condition D, False signal) was divided into two-minute periods. Data were analysed in seven consecutive 2-minute blocks. Pearson correlations were carried out on mean scores from the five groups against time block after the signal. These correlations encompassed scores from 2 minutes after the signal (as this is when I

expected behaviour would begin to be affected) up to 16 minutes (the last time period for which data were available for all five groups).

7.3 RESULTS

7.3.1 Effects of 'Signal Condition'

'Self-scratch', 'locomote' and 'forage' were significantly affected by 'Signal Condition' (see Table 7.7 and Figures 7.4, 7.5 and 7.6). For self-scratching, post-hoc pairwise *t*-tests indicated that rates in Conditions A and D were not significantly different, nor were those in Conditions B and C. Means were significantly higher in Condition A than in Conditions B and C when uncorrected p values were used, but there was no significant difference when the Bonferonni correction had been applied. Similarly, means for Condition D were significantly greater than those for Conditions B and C for uncorrected p values, but not significantly different following correction (see Table 7.8 and Figure 7.5).

Although there was a significant main effect of signal condition on locomotion, post-hoc tests did not reveal any significant differences between individual signal conditions, even before Bonferroni corrections were carried out (see Table 7.8 and Figure 7.5). There was significantly less foraging in the false signal condition than in any of the other conditions, both before and after the Bonferroni correction (see Table 7.8 and Figure 7.6).

Table 7.7 Repeated-measures ANOVA F and p values for effects of 'Signal Condition' and 'Time Period' on all behaviours

	Condition[1]		Period[2]	
Behaviour	**F**	**p**	**F**	**p**
Agonistic	3.43	0.05	1.96	0.55
Vocalise	2.68	0.09	0.18	0.84
Abnormal	1.40	0.29	1.11	0.38
Autogroom	1.22	0.34	7.02	<0.05*
Self-scratch	8.03	<0.01**	8.52	<0.01**
Locomote	4.27	<0.05*	34.19	<0.001***
Affiliative	2.63	0.10	22.04	<0.001***
Forage	68.59	<0.001***	496.85	<0.001***
Inactive	0.59	0.64	105.88	<0.001***

[1] d.f. =3,12; [2] d.f. =2,8
* p<0.05; ** p<0.01; *** p<0.001

Table 7.8 Post-hoc *t*-test *t* and p values for mean percentage sample points spent self-scratching, locomoting and foraging in each signal condition

Behaviour	**Comparison between conditions**	***t***	**p (uncorrected)**	**p (following Bonferonni correction)**
Self-scratch	A-B	3.74	<0.05*	0.12
	A-C	2.84	<0.05*	0.28
	A-D	1.11	0.33	1.00
	B-C	1.42	0.23	1.00
	B-D	4.11	<0.05*	0.09
	C-D	3.60	<0.05*	0.14
Locomote	A-B	0.06	0.95	1.00
	A-C	0.31	0.77	1.00
	A-D	2.25	0.09	0.53
	B-C	0.33	0.76	1.00
	B-D	2.41	0.07	0.44
	C-D	2.25	0.09	0.53
Forage	A-B	0.30	0.78	1.00
	A-C	0.56	0.61	1.00
	A-D	10.50	<0.001***	<0.001***
	B-C	0.58	0.59	1.00
	B-D	10.24	<0.001***	<0.001***
	C-D	9.80	<0.001***	<0.001***

All d.f. =4
* p<0.05; *** p<0.001

Figure 7.4 Mean percentage sample points spent in agonistic behavior, vocalising and in abnormal behaviours in each condition (bars represent Standard Errors)

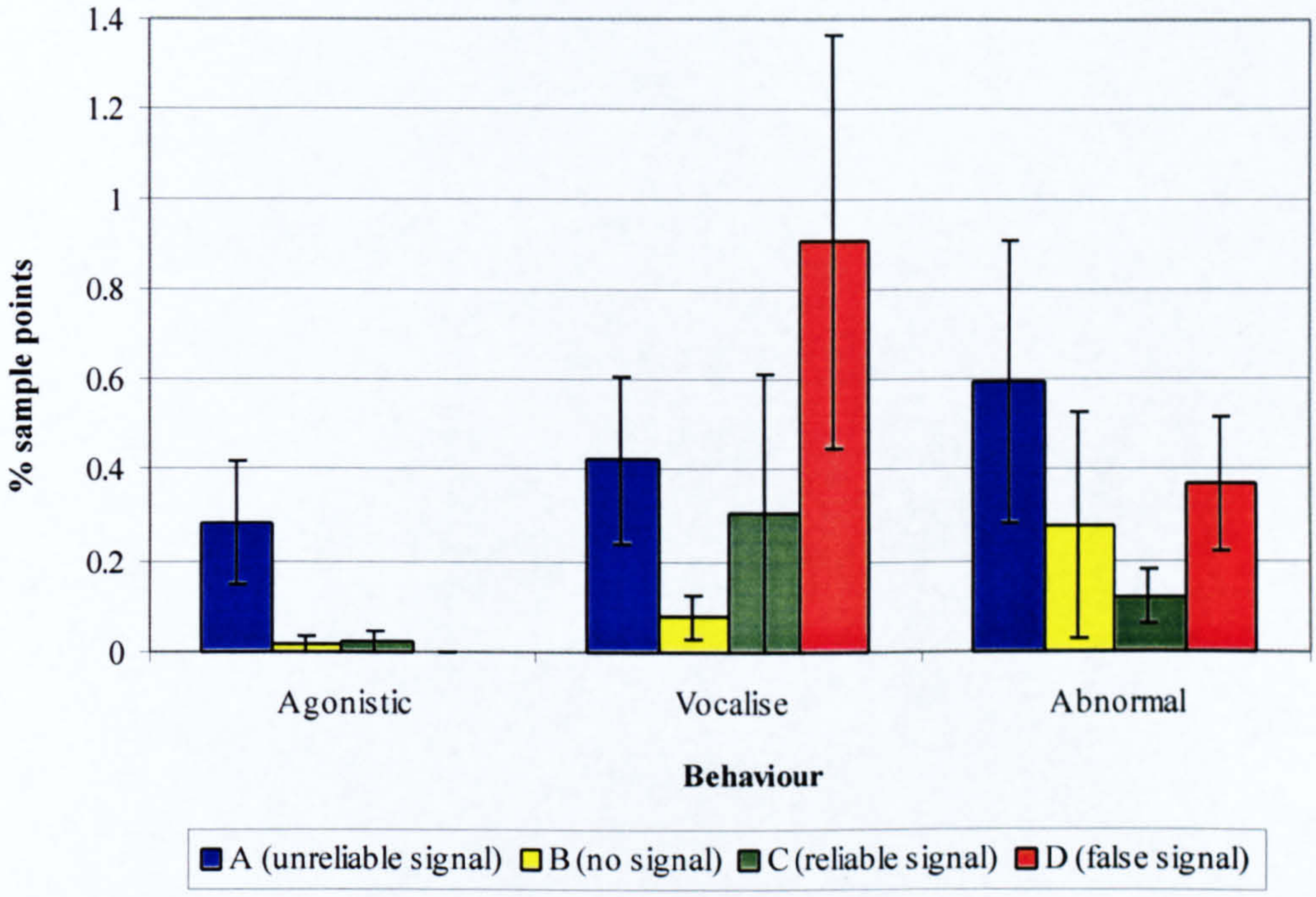
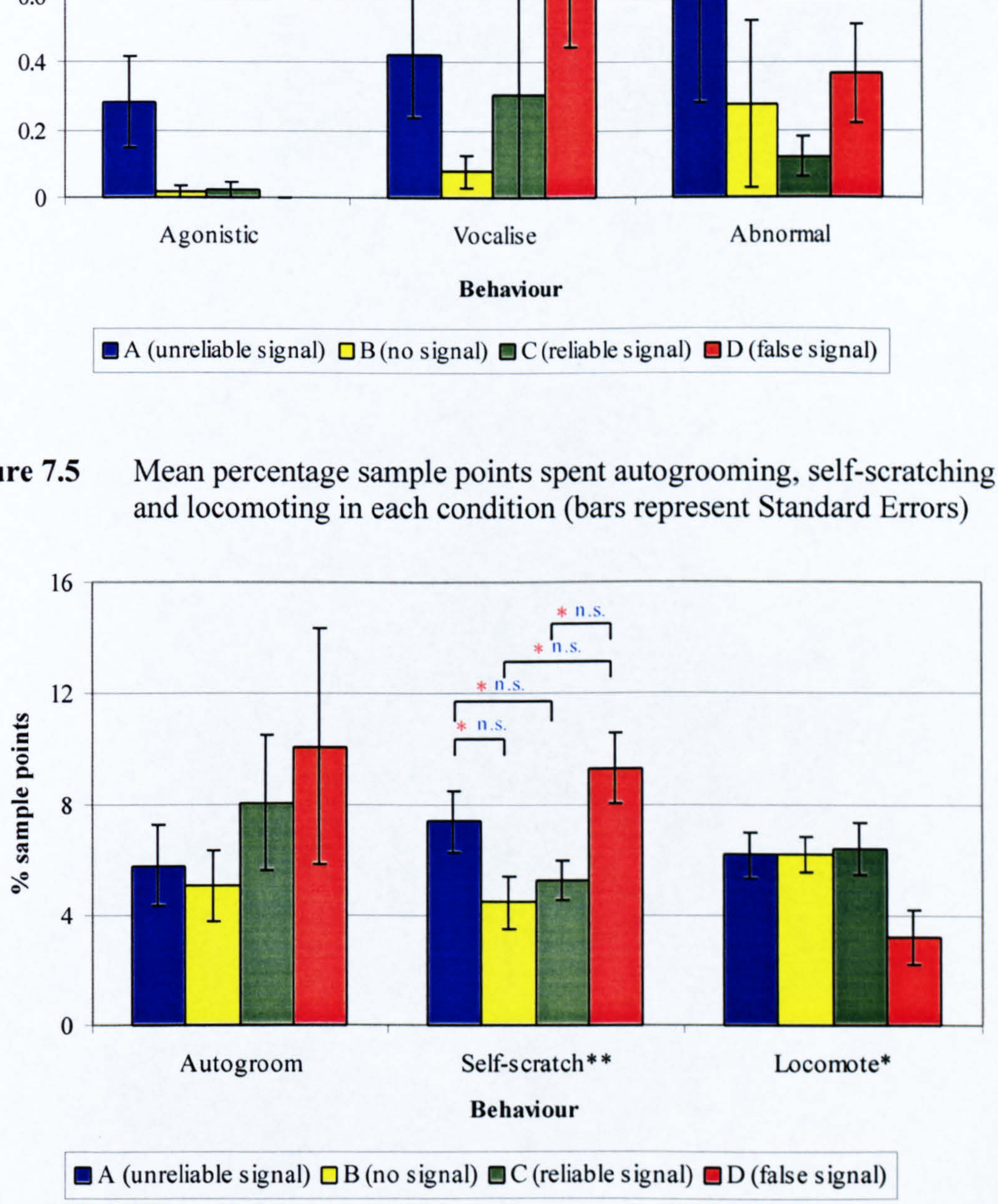
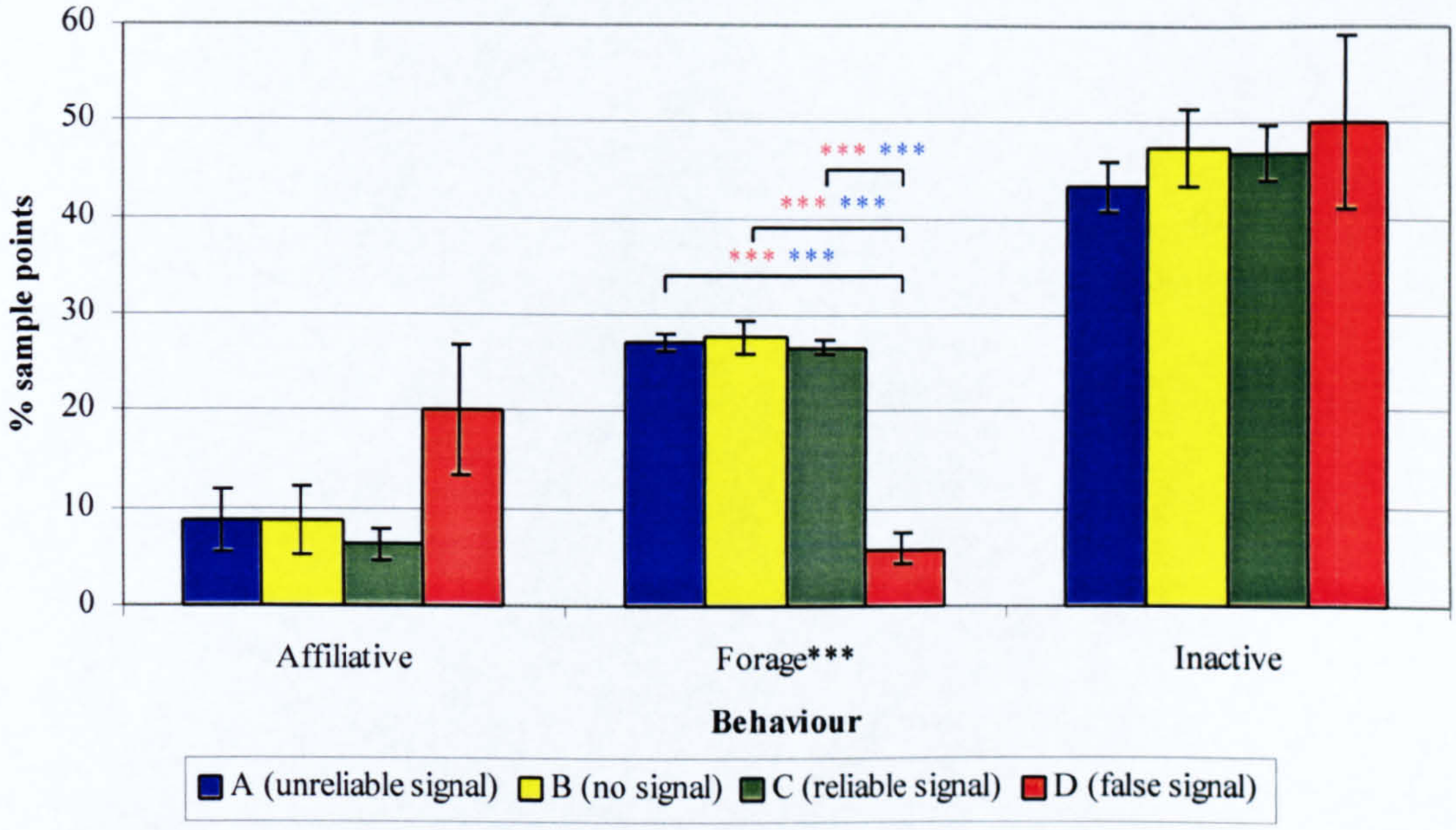


Figure 7.5 Mean percentage sample points spent autogrooming, self-scratching and locomoting in each condition (bars represent Standard Errors)



*p<0.05; ** p<0.01
Asterisks by x axis labels indicate significance of ANOVAs. Asterisks above boxes indicate significance of post-hoc pairwise *t*-tests (uncorrected values in red, corrected values in blue)

Figure 7.6 Mean percentage sample points spent in affiliative behaviour, foraging and inactive in each condition (bars represent Standard Errors)



*** p<0.001

Asterisks by x axis labels indicate significance of ANOVAs. Asterisks above boxes indicate significance of post-hoc pairwise *t*-tests (uncorrected values in red, corrected values in blue)

7.3.2 Effects of 'Time Period' of observation

'Autogroom', 'self-scratch', 'locomote', 'affiliative', 'forage' and 'inactive' were significantly affected by time period of the observation (see Table 7.7 and Figures 7.7, 7.8 and 7.9). Results of post-hoc *t*-tests are shown in Table 7.9. There was significantly more autogrooming in Period 1 than Period 3, when uncorrected p values were used. However, this difference was not significant when the Bonferroni correction had been applied (see Table 7.9 and Figure 7.8). Uncorrected p values indicated that there was significantly more self-scratching in Period 2 (i.e. after the signal but before the feed) than in either Periods 1 or 3 (before the signal and after the feed, respectively). However, again these differences were not significant when corrected p values were used (see Table 7.9 and Figure 7.8).

There was significantly more locomotion in Period 3 (after the feed) than in either Periods 1 or 2 (both before the feed, but pre- and post- signal respectively),

according to both corrected and uncorrected p values (see Table 7.9 and Figure 7.8). Rates of affiliative and inactive behaviours were significantly lower after the feed had been given than in either of the pre-feed conditions, irrespective of signal. This was true for both corrected and uncorrected p values (see Table 7.9 and Figure 7.9). Conversely, rates of foraging were significantly greater after the feed than in either of the pre-feed conditions (corrected and uncorrected values; see Table 7.9 and Figure 7.9).

Table 7.9 Post-hoc *t*-test *t* and p values for mean percentage sample points spent in each behaviour in each time period (only behaviours showing a significant main effect of 'time period' included)

Behaviour	Time period	*t*	p (uncorrected)	p (following Bonferroni correction)
Autogroom	1-2	0.26	0.81	1.00
	1-3	3.41	<0.05*	0.08
	2-3	2.56	0.06	0.19
Self-scratch	1-2	3.47	<0.05*	0.06
	1-3	1.70	0.17	0.50
	2-3	2.93	<0.05*	0.13
Locomote	1-2	0.18	0.87	1.00
	1-3	8.61	<0.001***	<0.001***
	2-3	6.50	<0.01**	<0.01**
Affiliative	1-2	1.59	0.19	0.56
	1-3	12.47	<0.001***	<0.001***
	2-3	4.05	<0.05*	<0.05*
Forage	1-2	0.65	0.55	1.00
	1-3	24.25	<0.001***	<0.001***
	2-3	25.01	<0.001***	<0.001***
Inactive	1-2	0.75	0.47	1.00
	1-3	10.39	<0.001***	<0.001***
	2-3	14.14	<0.001***	<0.001***

All d.f. = 4

* p<0.05; ** p<0.01; *** p<0.001

Figure 7.7 Mean percentage sample points spent vocalising and in agonistic and abnormal behaviours in the three time periods (bars represent Standard Errors)

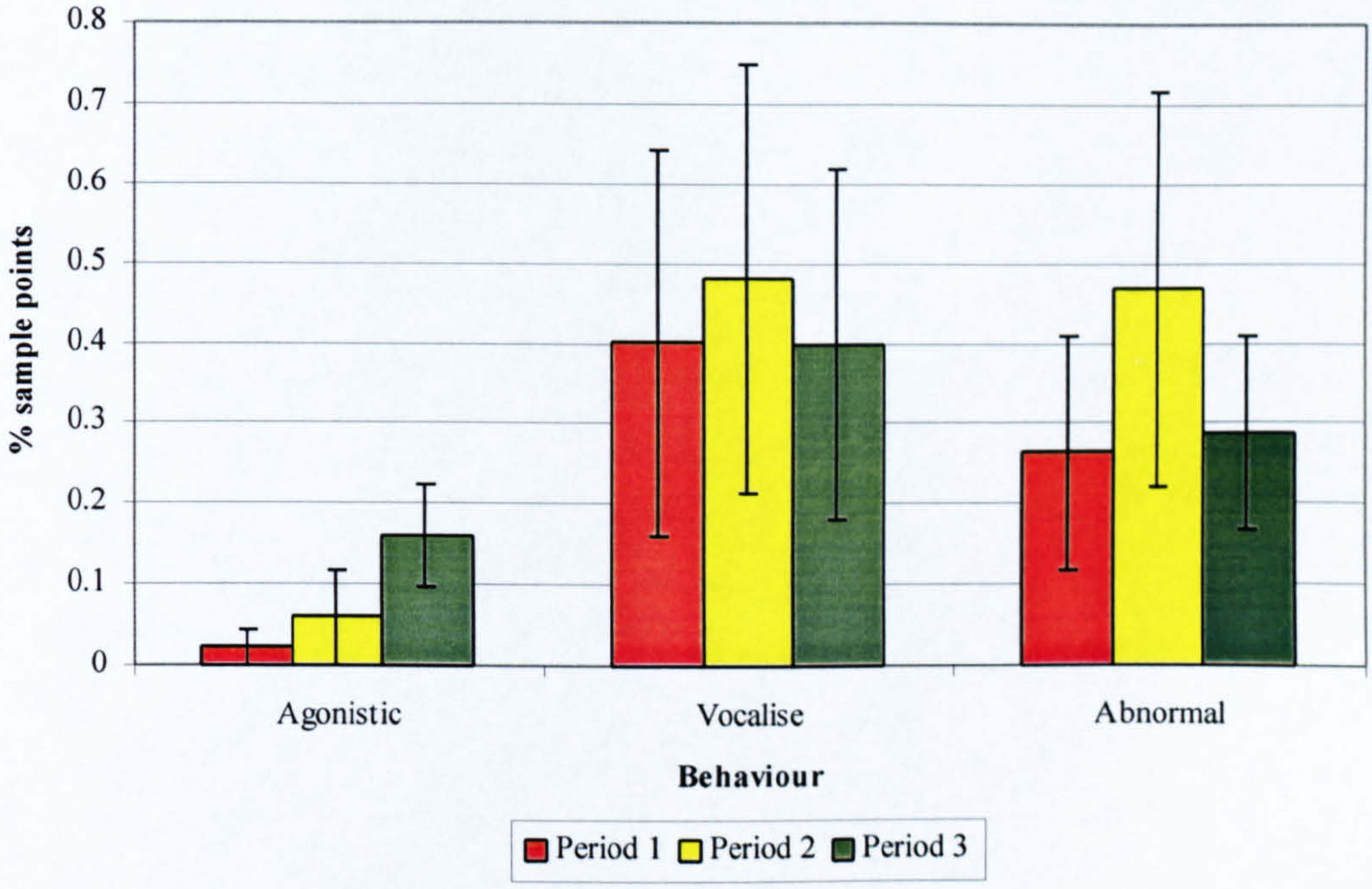
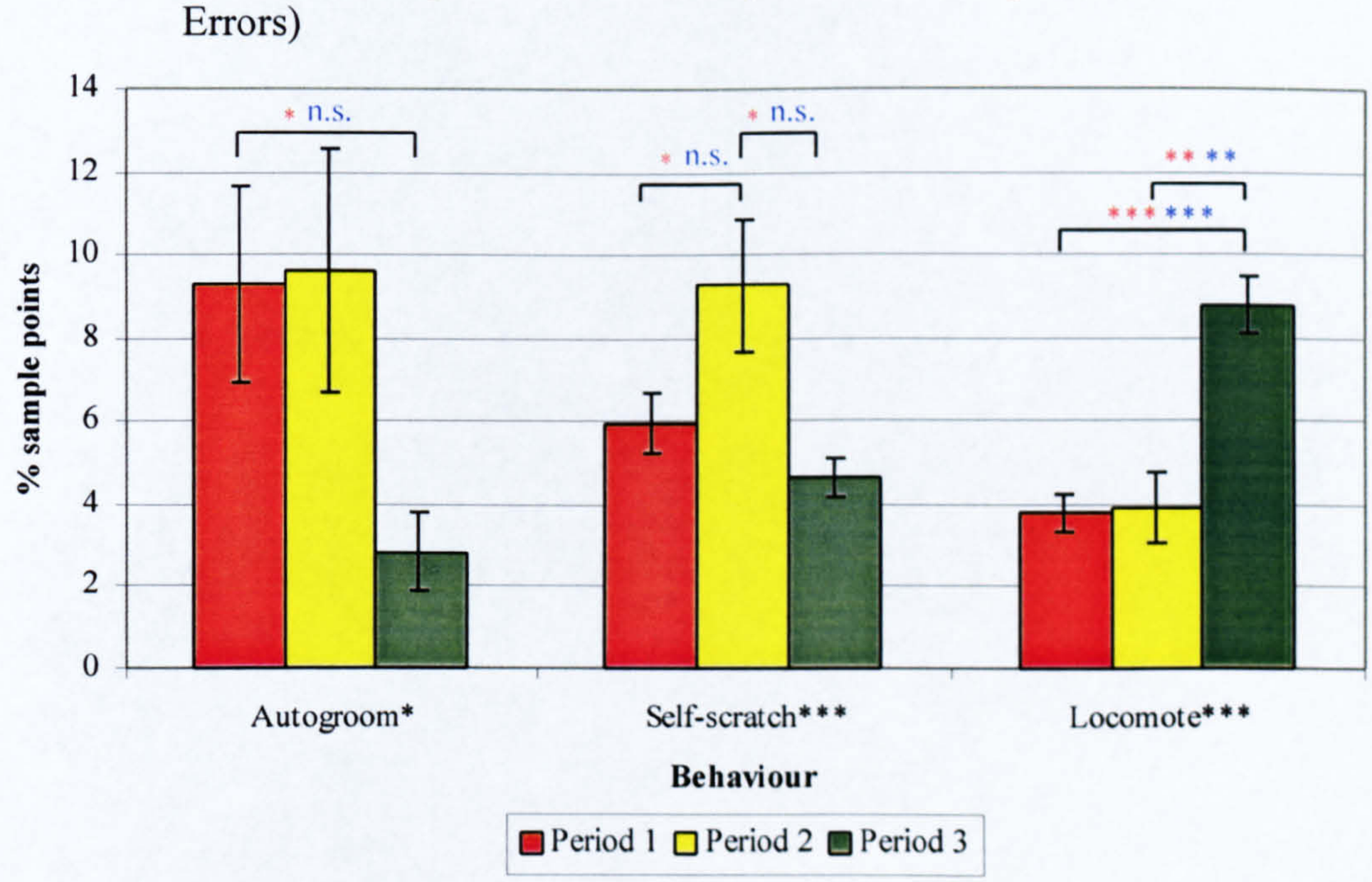
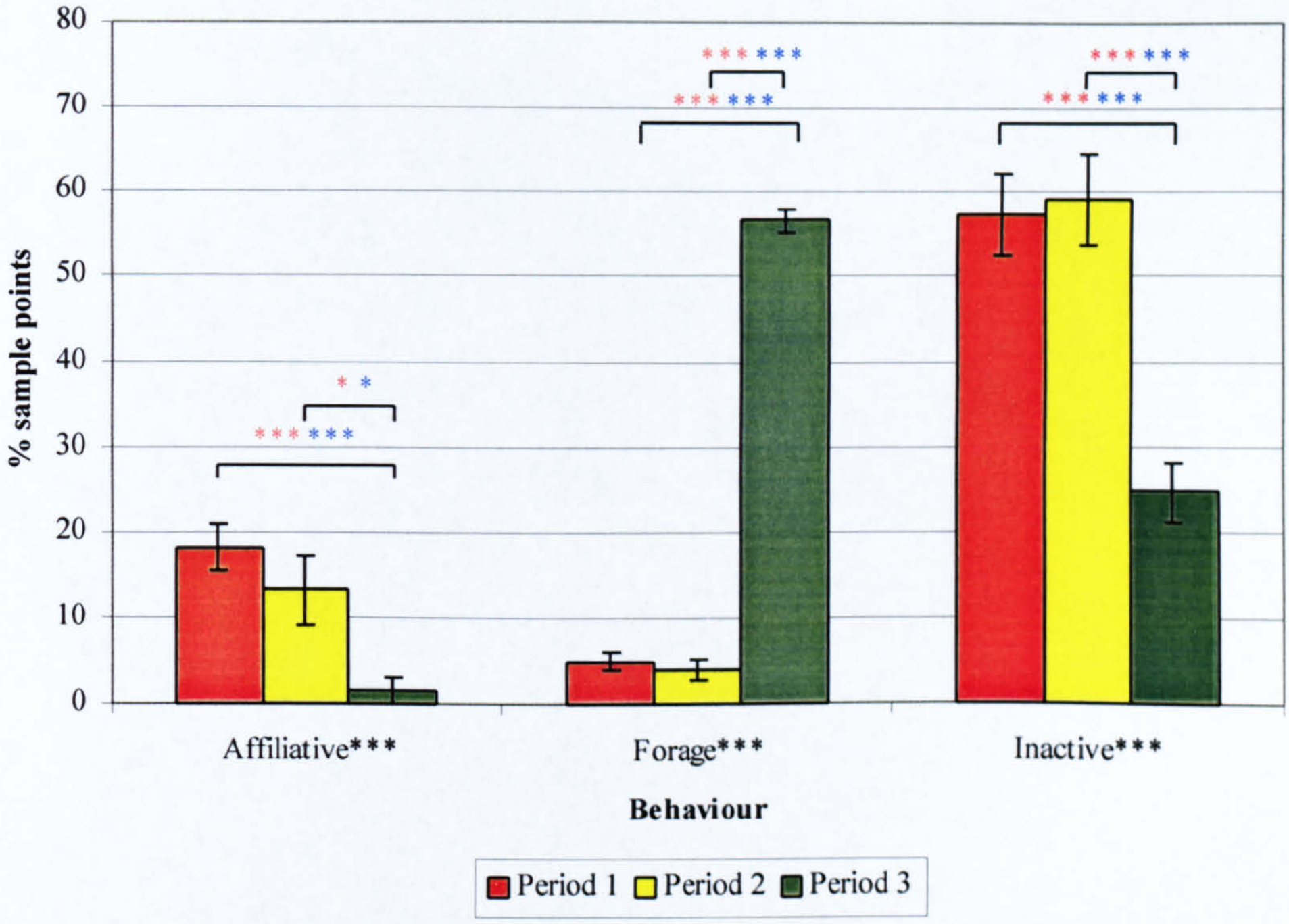


Figure 7.8 Mean percentage sample points spent autogrooming, self-scratching and locomoting in the three time periods (bars represent Standard Errors)



* $p<0.05$; ** $p<0.01$; *** $p<0.001$
Asterisks by x axis labels indicate significance of ANOVAs. Asterisks above boxes indicate significance of post-hoc pairwise *t*-tests (uncorrected values in red, corrected values in blue)

Figure 7.9 Mean percentage sample points spent in affiliative behaviour, foraging and inactive in the three time periods (bars represent Standard Errors)



* p<0.05; ** p<0.01; *** p<0.001
Asterisks by x axis labels indicate significance of ANOVAs. Asterisks above boxes indicate significance of post-hoc pairwise *t*-tests (uncorrected values in red, corrected values in blue)

7.3.3 Effects of interaction between 'Signal Condition' and 'Time Period' of observation

There were significant interactions between 'Signal Condition' and 'Time Period' for 'self-scratch', 'locomote', affiliative', 'forage' and 'inactive' (see Table 7.10). These significant interactions are described.

Table 7.10 Results of within-subjects ANOVAs for interactions between variables 'Signal Condition' and 'Time Period'

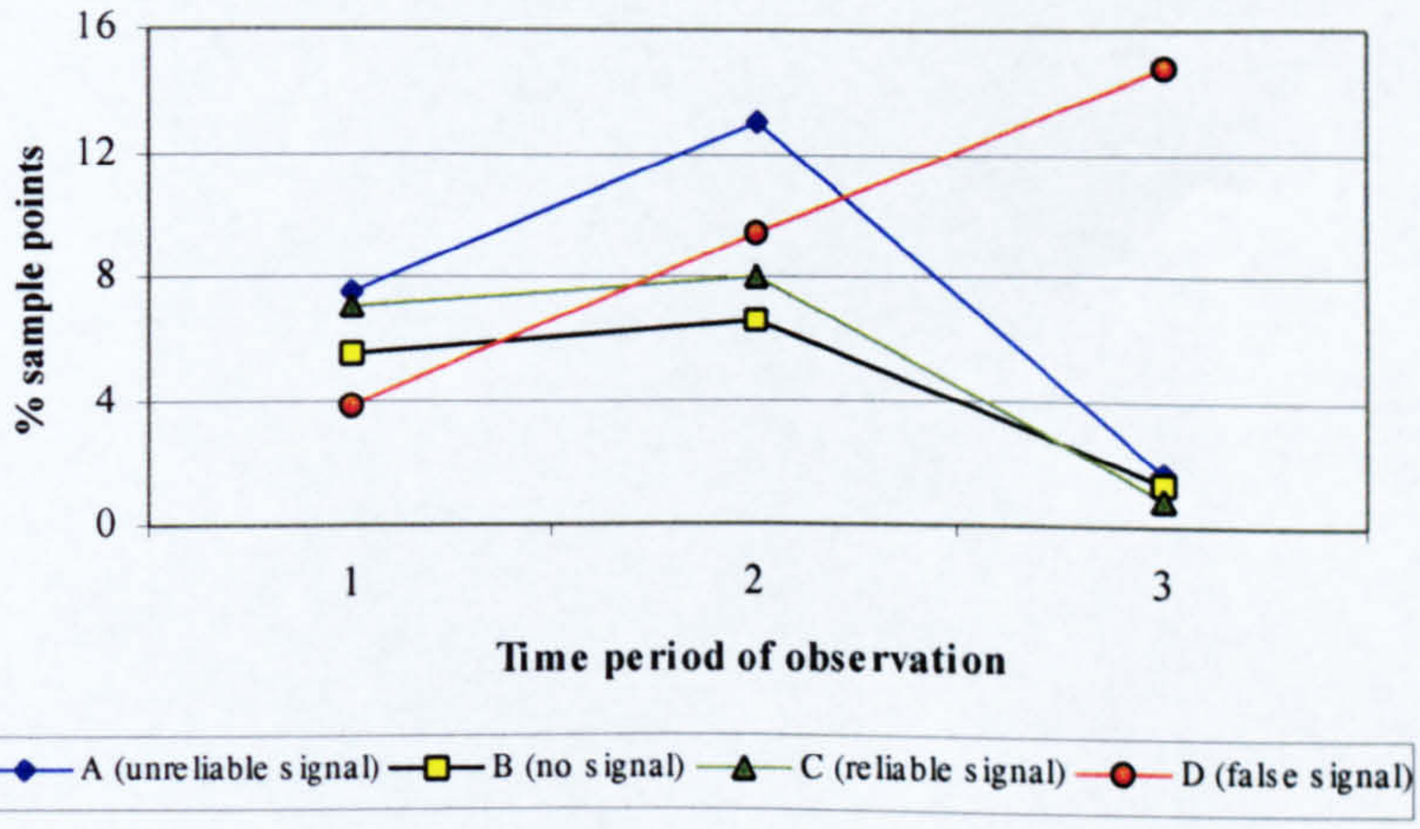
	Signal condition * Time period	
Behaviour	**F**	**p**
Agonistic	0.84	0.55
Vocalise	1.79	0.17
Abnormal	2.39	0.06
Autogroom	2.24	0.07
Self-scratch	15.89	<0.001***
Locomote	4.68	<0.01**
Affiliative	3.97	<0.01**
Forage	203.52	<0.001***
Inactive	32.04	<0.001***

df = 6,24

Self-scratch

Rates of self-scratching peaked at Time Period 2 for Condition A. Levels were relatively constant across Time Periods 1 and 2 for Conditions B and C. Rates of self-scratching reached a nadir at Time Period 3 for Conditions A, B and C, while those for Condition D increased throughout the observation period to reach a peak at Time Period 3 (see Figure 7.10).

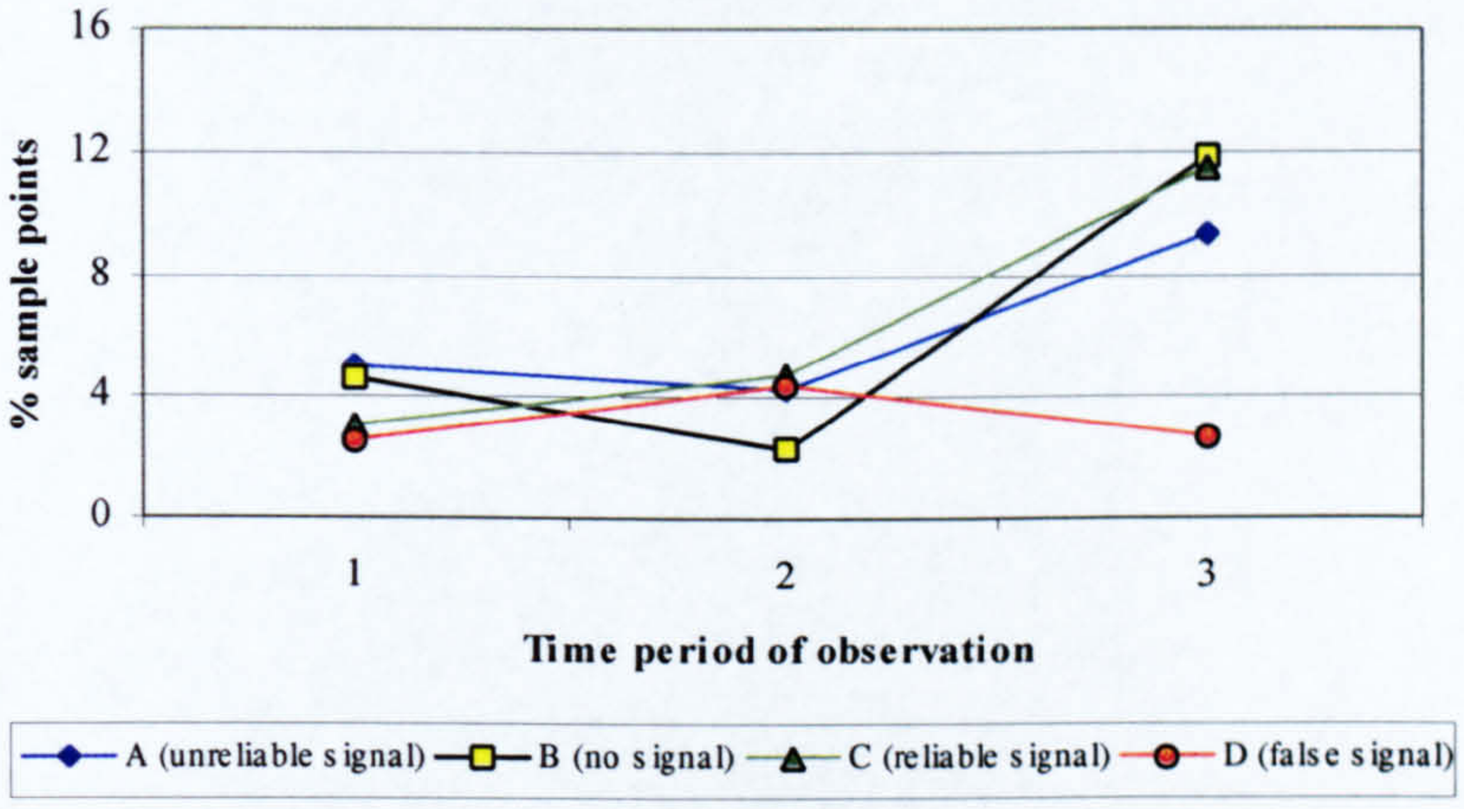
Figure 7.10 Graph of interaction between 'Signal Condition' and 'Time Period' for 'self-scratch'



Locomote

Rates of locomotion were fairly low in all conditions for Time Periods 1 and 2. At Time Period 3, however, rates increased for Conditions A, B and C. Rates for Condition D (false signal), however, remained low at all three time periods (see Figure 7.11).

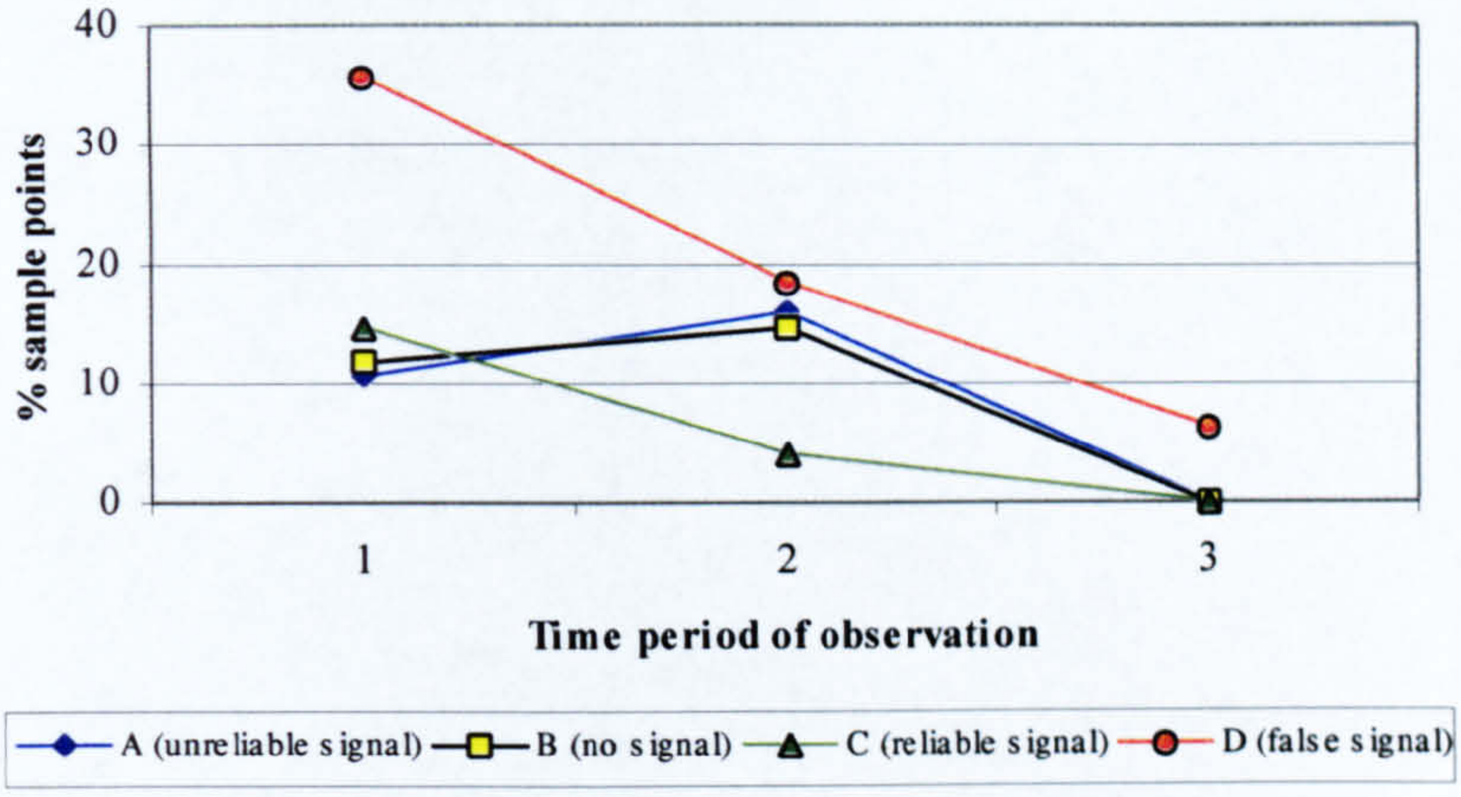
Figure 7.11 Graph of interaction between 'Signal Condition' and 'Time Period' for 'Locomote'



Affiliative

Rates of affiliative behaviour were fairly low throughout the observation period for Conditions A, B and C. At Time Period 3, rates decreased to zero for each of these three conditions. By contrast, rates were much higher at Time Period 1 for Condition D, and decreased throughout the observation period, although not dropping as far as zero in Time Period 3 (see Figure 7.12).

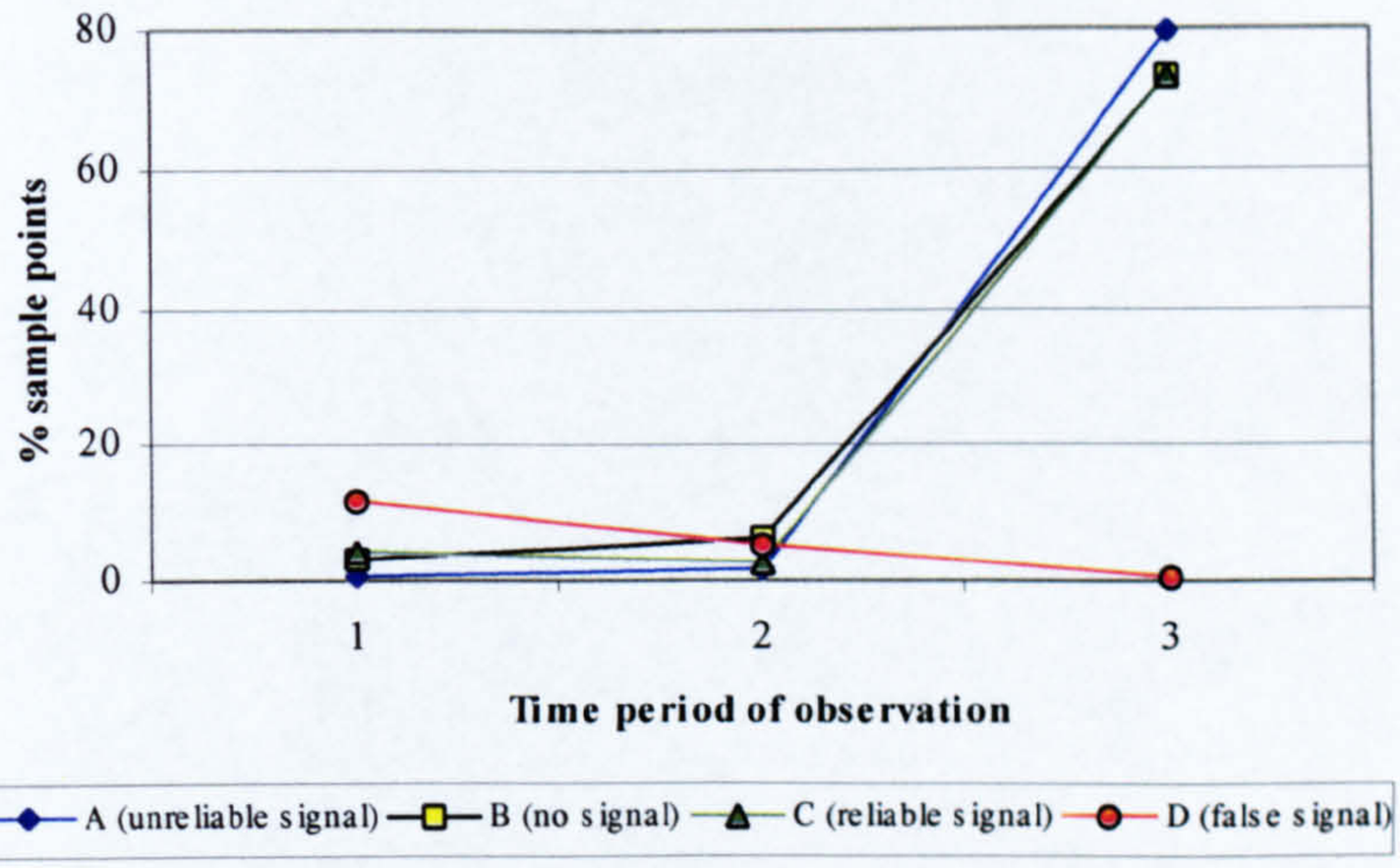
Figure 7.12 Graph of interaction between 'Signal Condition' and 'Time Period' for 'Affiliative'



Forage

Rates of foraging were very low for all conditions at Time Periods 1 and 2. However, while rates increased dramatically for Conditions A, B and C at Time Period 3, at this period rates were zero for Condition D (see Figure 7.13).

Figure 7.13 Graph of interaction between 'Signal Condition' and 'Time Period' for 'Forage'

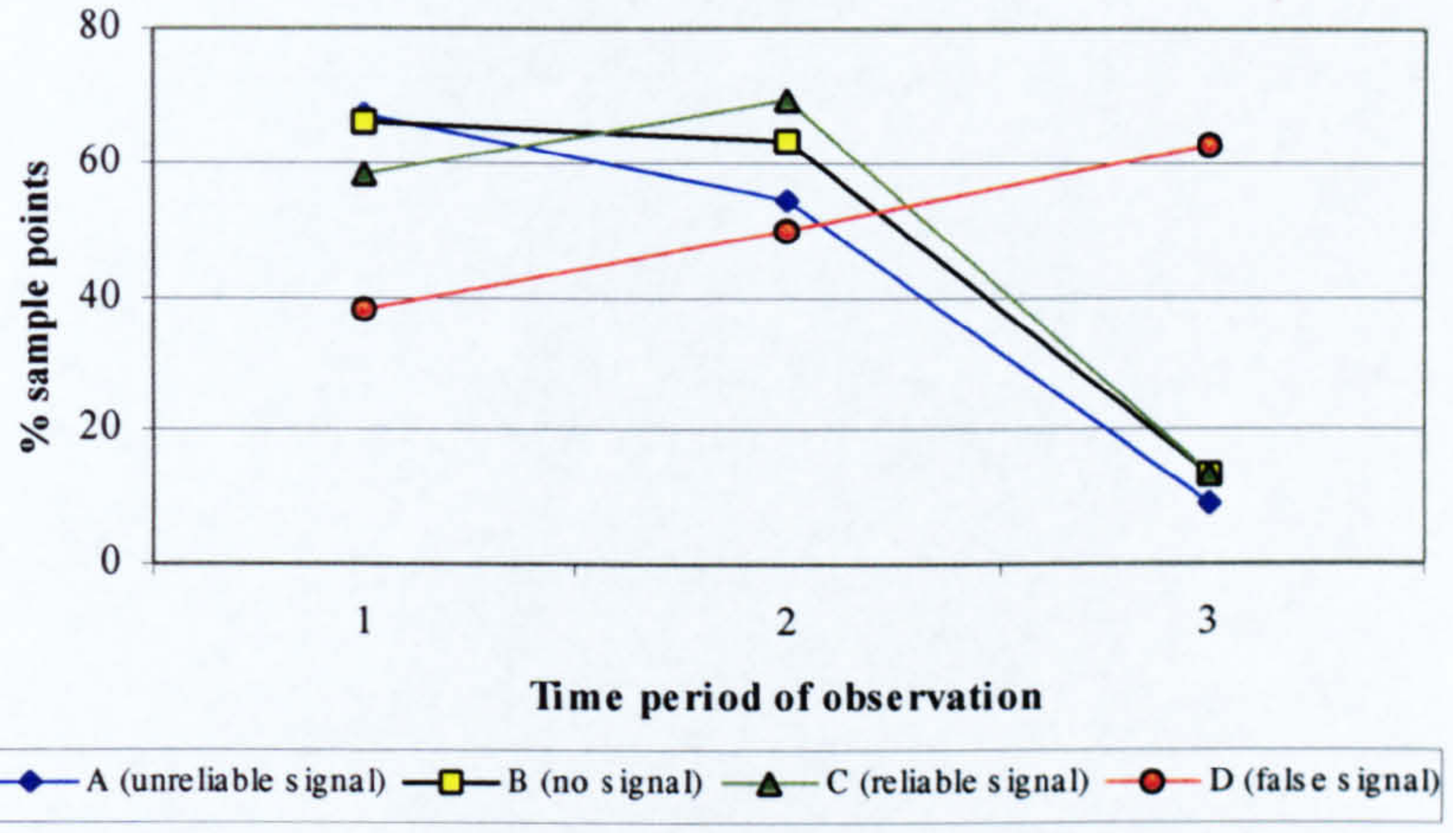


Inactive

Levels of inactivity were fairly high at Time Periods 1 and 2 for Conditions A, B and C. They decreased markedly at Time Period 3 for each of these three

conditions, however. In contrast, for Condition D, rates of inactivity were intermediate at Time Period 1, and increased steadily throughout the observation period (see Figure 7.14).

Figure 7.14 Graph of interaction between 'Signal Condition' and 'Time Period' for 'Inactive'



7.3.4 Effects of time after the false signal in the absence of food

Pearson correlations carried out on behaviours between 2 and 14 minutes after the false signal showed an increase in abnormal and inactive behaviour with time following the false signal. Affiliative behaviour, autogrooming and vocalising showed decreases with time following the false signal. Foraging, locomotion and self-scratching were unaffected by increasing time following the false signal (see Table 7.12 and Figure 7.15).

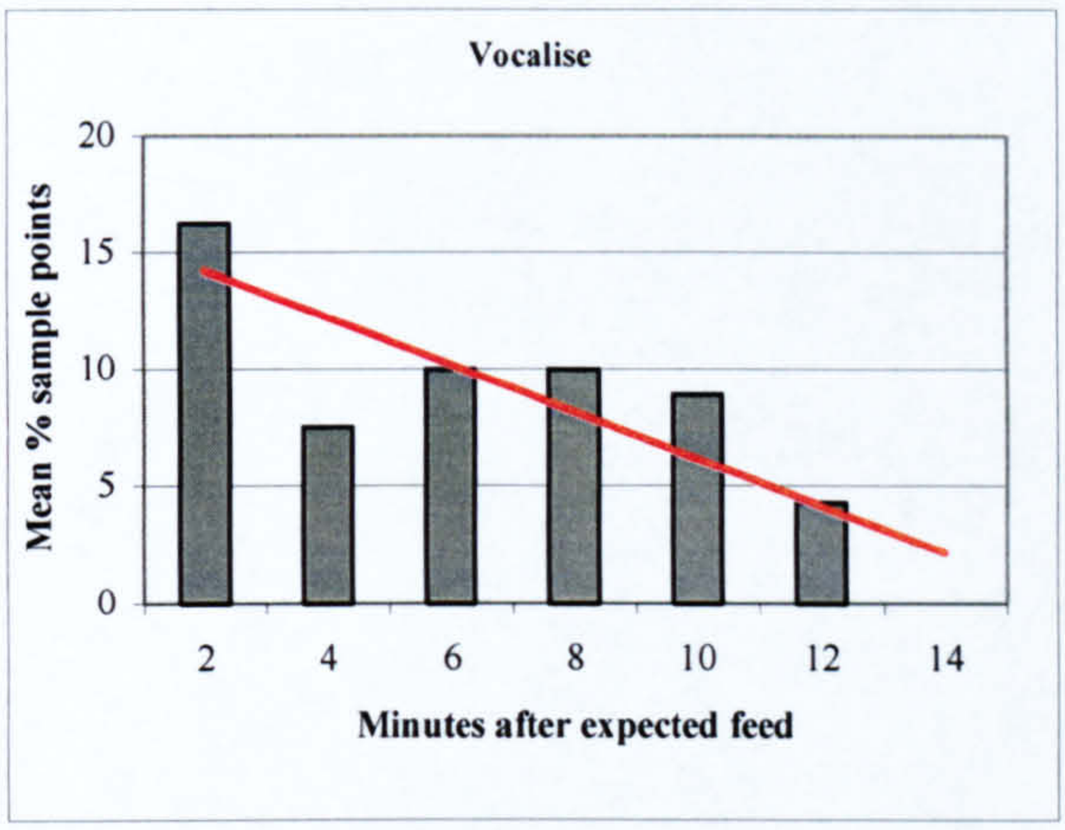
Table 7.12 Pearson correlation coefficients and p values for the effects of time after the false signal on all behaviours

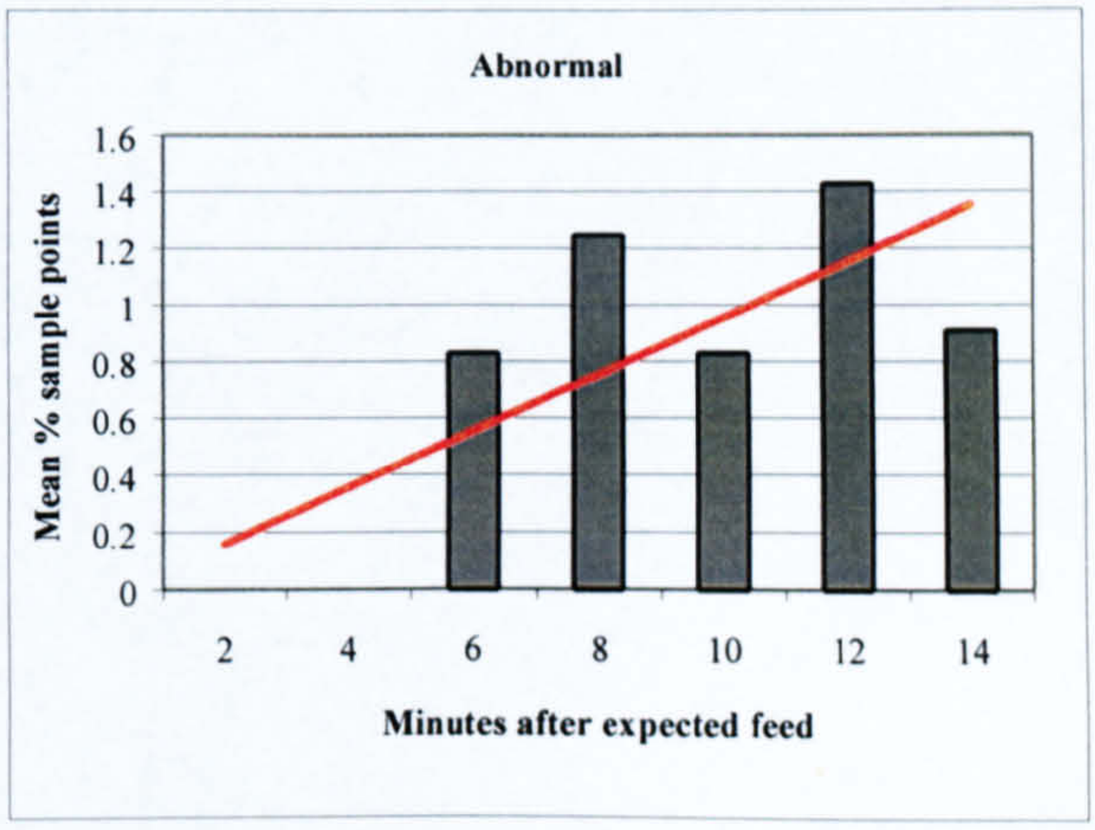
Behaviour	r	p (two-tailed)
Agonistic	-	-
Vocalise	-0.855	<0.05*
Abnormal	0.771	<0.05*
Autogroom	-0.778	<0.05*
Self-scratch	0.448	0.313
Locomote	0.133	0.777
Affiliative	-0.855	<0.05*
Forage	-0.700	0.080
Inactive	0.852	<0.05*

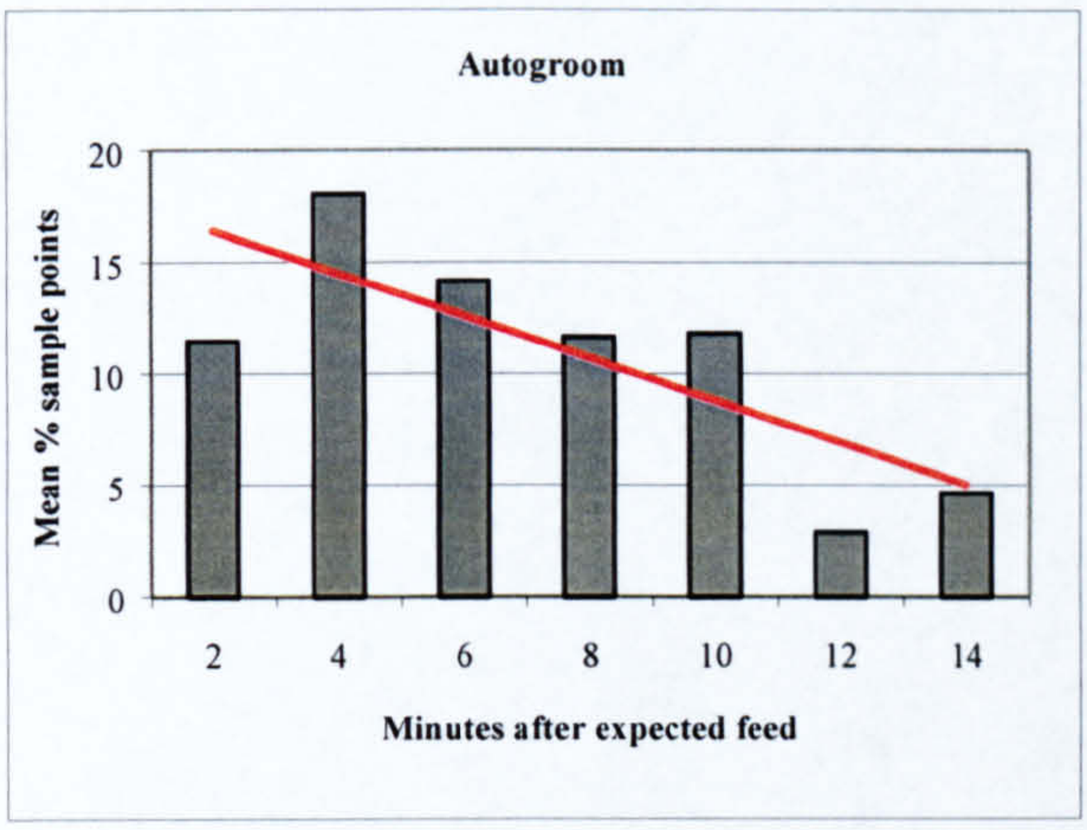
All n=7

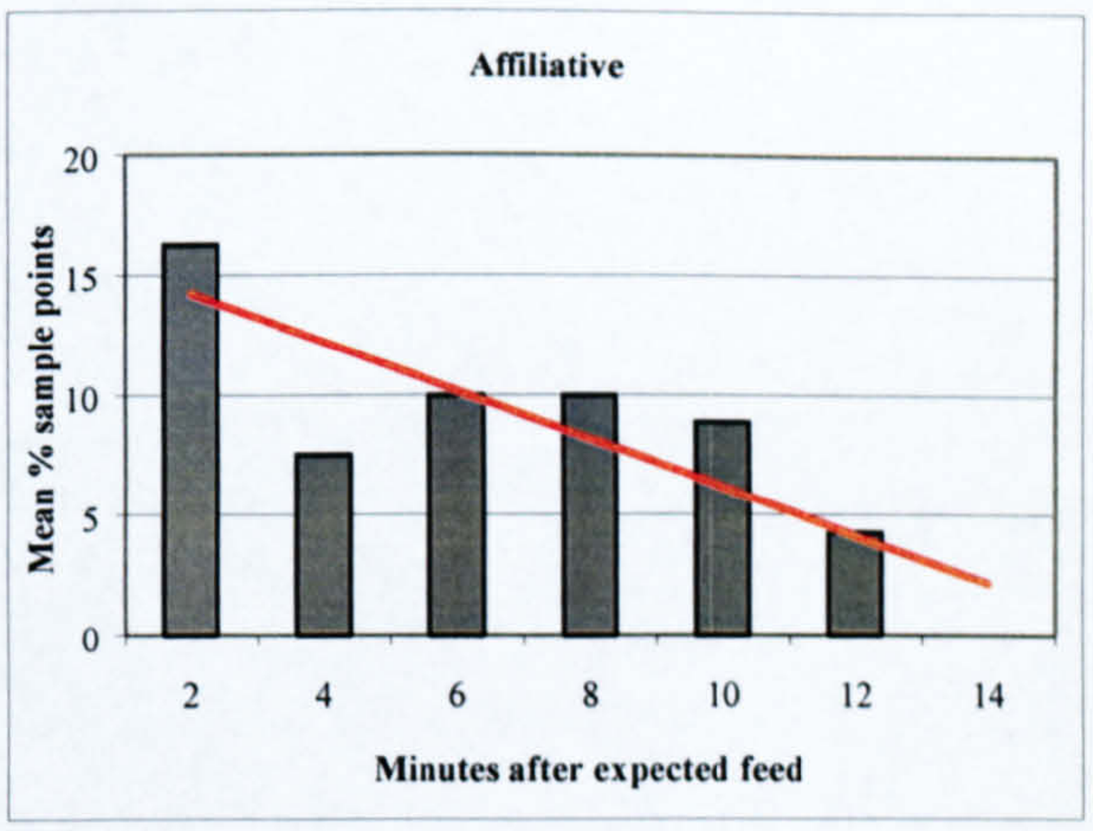
*p<0.05

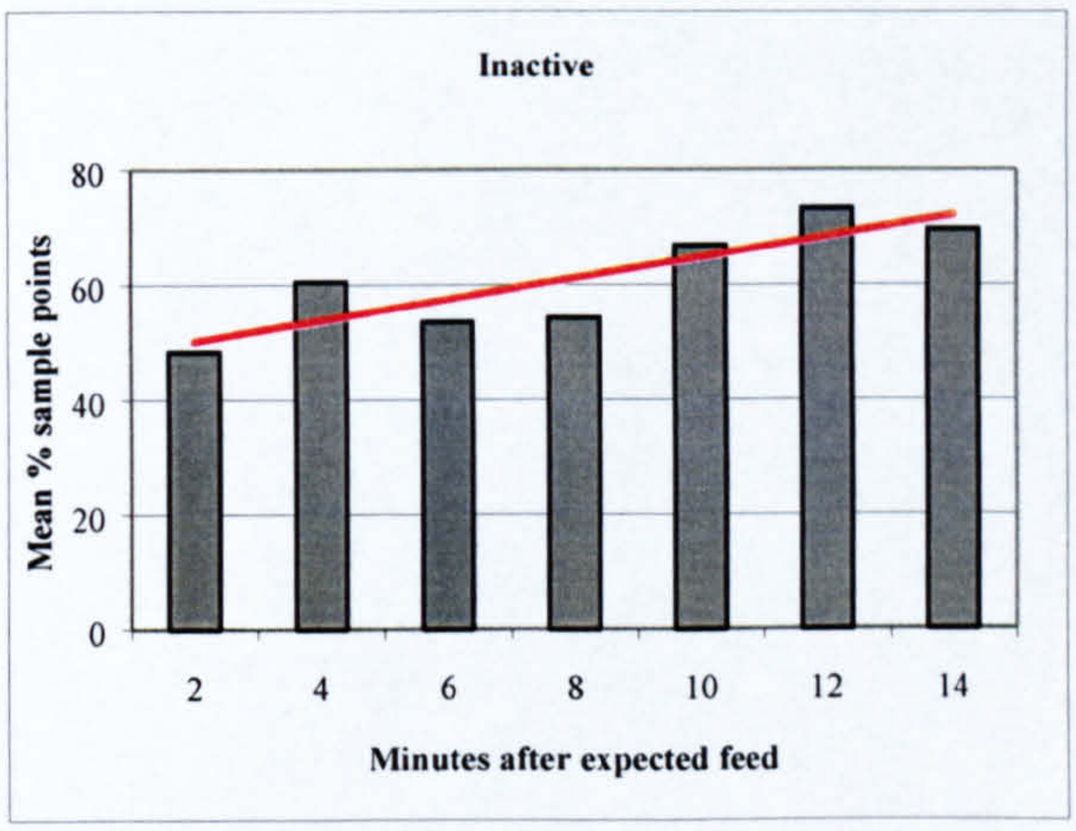
Figure 7.15 Graphs of mean percentage sample points spent in each behaviour, at 2-minute intervals following the signal, for which the Pearson correlation was significant. Data are included for periods 2 to 14 minutes after the expected feed. Trendlines representing the association between the variables have been added in red.











7.4 DISCUSSION

Effects of 'Signal condition'

Self-scratching, locomotion and foraging were significantly affected by signal reliability. Probably the most important and interesting finding of this study was that rates of self-scratching were affected in a similar way in response to feeding predictability as they were in the study described in Chapter 5. Scratching is thought to be a reliable indicator of anxiety both in primates generally (see Chapter 1 and review by Maestripieri *et al*, 1992) and in this species in particular (see Introduction to this chapter). For this reason, we may be fairly confident that the observed changes in scratching, in response to experimental manipulations, reflect changes in anxiety and therefore welfare within the study population. However, it should also be borne in mind that differences between the conditions were only significant before the Bonferroni correction had been applied to the relevant p values. Although the Bonferroni correction may be excessively conservative, possibly leading to Type II errors where a real effect is missed, use of uncorrected p values may lead to Type I errors – achieving a false positive effect (Everitt, 1996). In an attempt to avoid these two extremes, uncorrected p values are tentatively accepted here, but must always be viewed with a degree of caution.

In common with the marmosets in Chapter 5, the stump-tailed macaques showed increased rates of self-scratching in response to an unreliable signal preceding food delivery (*i.e.* when the animals were not fed despite having heard feeding-related sounds). The results of this study were therefore in line with the main findings of the initial one. Additionally, in this study it was shown that a false signal (*i.e.* an unreliable signal that had previously been reliable) resulted in increased scratching. Rates of scratching did not differ between the 'false signal' condition and the 'unreliable signal' condition, suggesting that unreliable signals are equally stressful regardless of the number of unreliable signals the animal has previously been exposed to.

Locomotion and foraging were also significantly affected by signal condition. However, post-hoc tests revealed no significant differences between individual conditions for locomotion, indicating that the significance of the original ANOVA was marginal. It is therefore difficult to draw any meaningful conclusions from these data. Nevertheless, the pattern seen for locomotion is very similar to that of foraging. Post-hoc tests revealed that there was significantly less foraging in Condition D (false signal) than in any of the other conditions. This is almost certainly due to the increased availability of food in Conditions A-C compared with Condition D. In the first three signal conditions, the animals were fed during the observation period, yet in the fourth condition they did not receive any food. It is also likely that the patterns seen for locomotion were due to the same factor, with the animals moving around the enclosure in order to collect food that had been scattered around.

Effects of 'Time Period' of observation

It is unsurprising that the 'Time Period' of the observation, in relation to the signal and the feeding event, significantly affected behaviour. Rates of foraging and locomotion were higher after the feed than before, as the animals moved around the enclosure where the food had been scattered, and foraged for it. In common with the data associated with signal condition, similar patterns were seen for foraging and locomotion in relation to the time period of the study. This again supports the idea that these two behaviours were influenced by the same factor, namely food availability.

The only behaviour, apart from foraging and locomotion, that appeared to increase after feeding was agonistic behaviour, although this trend was not significant. A semi-free ranging group of stumptailed macaques showed increased agonistic behaviours such as threats, aggression and submission when eating provisioned food, less when not eating food, and least when eating natural foods

(Jolly & Rasmussen, 1991). The authors suggest that this may be due to the clumped high-density nature of the artificially provisioned food in comparison with the lower-density wider dispersal of the natural food. The macaques in this study were fed in the 'gang room', with an area of around 13.5m^2. This is a relatively tiny area in comparison to their wild habitat, where they may range 3000m in a day (Bertrand, 1969). The food presented in the 'gang room' could therefore be considered 'clumped' and 'high density', which may account for the apparently increased levels of agonism on its delivery.

Rates of affiliative and inactive behaviour were lower after the feed than before it. The fact that these differences remained significant following the Bonferroni correction indicates that these results are extremely robust. A similar pattern was seen for autogrooming, with significantly less autogrooming in Period 3 than Period 1. However, this difference became non-significant when the Bonferroni correction was applied, which decreases confidence in the result. This lower level of significance is likely to be due to the large variability of the data, as indicated by the large standard error bars. It is possible that the reduction in any of these three behaviours (*i.e.* affiliative, inactive and autogrooming) was because tension was alleviated by the presentation of food. However, it is more likely to be due to the fact that the animals were so busy foraging (spending around 66% of sample points in a combination of foraging and locomotion) that they had little time to do anything else.

There was a significant effect of time period of observation on self-scratching, with significantly lower rates of this behaviour in Periods 1 (before the signal) and 3 (after the feed) than Period 2 (after the signal but before the food). This suggests that tension was highest in the macaques in the short period after the signal (in Conditions A, C and D; no signal was given in Condition B) and before the feed (in Conditions A, B and C; no feed was given in Condition D). However, adjusting the p values to allow for the Bonferroni correction rendered these

differences non-significant; once again, therefore, caution must be maintained when interpreting the results. It is likely that both internal (*i.e.* hunger) and external cues (*i.e.* the experimental signal, as well as possibly other signals that could not be controlled, such as staff carrying out routine jobs at certain times) led to increased tension and frustration as feeding time approached. Food delivery is likely to have effectively negated these effects.

It is also interesting to note that the same pattern was seen for self-scratching in both the marmoset studies (Chapters 5 and 6; see Figures 5.7 and 6.7). However, these results were non-significant in the signalled predictability study (Chapter 5). Only a significant main effect of 'Time Period' was found in the temporal predictability study (Chapter 6), with no significant differences between individual time periods.

Vocalising and abnormal behaviour were not significantly affected by the time period of the observation. They both however showed the same trend as was seen for self-scratching, which was higher in Period 2 (post-signal, pre-feed) than either Period 1 (pre-signal) or Period 3 (post-feed). The non-significance of the result for these behaviours is likely to be due to the fact that rates of these behaviours were very low to begin with, and they were also very variable, as is shown by the large Standard Error bars on the relevant graphs, making significance less likely.

Effects of interaction between 'Signal Condition' and 'Time Period' of observation

Five behaviours showed significant interactions between 'Signal Condition' and 'Time Period'. These were self-scratching, locomotion, affiliative behaviour, foraging and inactive behaviour. The significant interaction between the variables for self-scratching indicates that some of the conclusions drawn earlier in the discussion may have been over simplified. For example, a significant main effect was originally found for 'Time Period', with higher rates of the behaviour seen in

Period 2 than either Periods 1 or 3 (although not significantly so when Bonferroni correction had been applied). However, the interaction shows that this was only the case for Condition A, when the naturally occurring, unreliable signal was used. This suggests, in common with Chapter 5, that the presence of an unreliable signal results in an increase of stress-related behaviour, which is sustained until food is delivered. When animals received either no signal or a reliable signal they also showed a reduction in self-scratching when food was delivered, but this differed from the unreliable signal condition in that they did not show this increase at Time Period 2.

When exposed to the false signal (Condition D), animals showed a similar pattern in rates of self-scratching over Periods 1 and 2 as when exposed to the unreliable signal (Condition A). Rates were however slightly lower in the false signal condition than the unreliable signal condition. This may have been due to the fact that observations in the false signal condition were carried out significantly earlier than those in the other three conditions. It is possible that rates of stress-related behaviours such as self-scratching might increase as feeding time approached, irrelevant of signal absence or presence. However, at Time Period 3, rates continued to increase for Condition D. They reached a level higher than was seen for any other condition at any point of the study, indicating that stress levels were highest at this period. It is important to remember that food delivery was not actually late in temporal terms at this point, as it was not given at a significantly different time for any of the four conditions. Instead, the increase in self-scratching is likely to have been due to the non-delivery of food following the signal. This is likely to have caused frustration, a 'reaction elicited by nonreward after a number of prior rewards' (Amsel, 1958, p. 103).

Interactions between the variables resulted in similar patterns for both locomotion and foraging. For both these behaviours, levels were low during the first two time periods for all conditions. However, at Time Period 3, levels

increased for those conditions that received food (*i.e.* Conditions A, B and C). In the case of foraging, this was clearly due to the food delivery. The close association between the patterns seen for this behaviour and that of locomotion indicate that food availability is likely to be an important factor relating to levels of locomotion also, with animals moving around the enclosure to collect food items that had been scattered around. Unsurprisingly, levels did not increase at Time Period 3 for Condition D, where no food was delivered.

Rates of affiliative behaviour were fairly low during Time Periods 1 and 2 for Conditions A, B and C. They decreased to zero for all these conditions at Time Period 3, when food was delivered. However, rates were much higher at Time Period 1 for Condition D than for any other condition or time period. It is difficult to account for this high level of affiliative behaviour, which almost exclusively consisted of allogrooming.

There is controversy over exactly what increased levels of social grooming may mean in terms of primate welfare. For example, studies have shown (Boccia *et al*, 1989; Nieuwenhuijsen & de Waal, 1992; Schino *et al*, 1988) that allogrooming may function as a tension reduction mechanism in primates. Receiving grooming appears to be associated with a reduction in heart rate in the pigtail macaque *(Macaca nemestrina)* (Boccia *et al*, 1989). Increased levels of grooming may therefore be interpreted as indicative of high levels of tension. However, grooming is thought to be important in the development and maintenance of social bonds and group cohesion in primates (*e.g.* Dunbar, 1991; Sade, 1965; Schino *et al*, 1988). Increased levels of grooming may therefore also be considered desirable in captive primates as they may indicate improved social functioning of the group.

The major difference between Condition D and the other three conditions was that animals did not receive food during the observation period in Condition D. However, another important difference between Condition D and the other conditions was that observations were made significantly earlier in Condition D.

This was so that a false signal could be sounded without having to delay the feed, which in itself may have been stressful for the animals, irrespective of any signal (Waitt & Buchanan-Smith, 2001). This would therefore have been a potentially confounding factor in the study. It is possible that the high rates of affiliative behaviour seen during Time Period 1 for Condition D may simply have reflected the fact that the observations were made significantly earlier than any of the others in the study. Affiliative behaviour may, therefore, have been at similar levels to this for all conditions irrespective of signal. It is, however, impossible to ascertain whether this was the case as sufficient matched observations were not made at this time period for any of the other conditions.

Additionally, only two observations were made of each group in Condition D, compared with four in each of the other conditions. Time budgets were constructed from means of all the observation sessions for each group in each condition; hence, each observation session made up 25% of the data for Conditions A-C, but 50% for Condition D. Any unusual behaviours seen in a single observation period would have been more obvious in Condition D, as they would have been less 'diluted' by the extra observation periods.

The time of the observation may also have been a causal factor in the interaction seen for inactive behaviour. Here, levels of inactivity were fairly high for Conditions A, B and C for Time Periods 1 and 2. Levels fell sharply in these conditions at Time Period 3, when food was provided and levels of foraging increased.

Levels of inactivity were lower in Condition D at Time Period 1, increasing to levels similar to those seen before feeding for the other conditions at Time Periods 2 and 3. Again, inactivity may have been lower across all conditions at the time that the observations were made in Time Period 1 for Condition D. The lack of corresponding data at this time mean that this remains no more than an hypothesis. An increase in inactivity before feeding time has however been reported for

chimpanzees (Bloomsmith & Lambert, 1985), and the same population of stump-tailed macaques as was used in the present study (Waitt & Buchanan-Smith, 2001). Both these studies described the animals as appearing to sit around waiting for their food to arrive. Increased levels of inactivity may be undesirable in many species of captive primates that are susceptible to weight gain and obesity, such as the stump-tailed macaque. This contrasts with the common marmoset, the focus of the previous three chapters of the thesis, in which increased levels of inactivity appear to be indicative of reduced stress. Weight gain is not seen as such a problem in this species (Morris, pers. comm.).

Effects of time following the false signal in the absence of food

The increase in abnormal behaviour (which generally consisted of self-aggressive behaviours and self-clasping) with time following the false signal could be interpreted as an indication that anxiety increases the longer an expected meal does not arrive. This would suggest that delays to feeding after the activation of an otherwise reliable signal should be kept as short as possible. The increase in inactivity seen with time following the false signal would suggest the same thing, simply because this behavioural change is generally considered undesirable in captive primates (e.g. Bloomsmith & Lambert, 1985; Dittrich, 1984; Tripp, 1985). There tends to be increased inactivity in captive primates in comparison with the wild activity budget, and the obesity often seen in captivity is likely to be, at least in part, due to this factor (Bloomsmith & Lambert, 1985).

However, in contrast to these changes, other behaviours thought to be indicative of increased anxiety, namely autogrooming and vocalising, *decrease* with increasing time following the false signal. This is in contrast to another study on the same population of animals, in which vocalisation rates were found to increase before feeding, with these increased rates being maintained when feeding was delayed (Waitt & Buchanan-Smith, 2001). Another behaviour sometimes

considered desirable yet associated with excitement and tension in this species, allogrooming, also decreases with increasing time after the false signal. These observations seem to suggest that tension is greatest immediately following the non-appearance of the expected food, and decreases following this. The practical implication of this would be that welfare is negatively affected if food is not delivered at exactly the same time after the reliable signal as has been conditioned. Nevertheless, if there is a delay, it becomes less urgent with increasing time to deliver the food immediately, as tension and anxiety decrease over time following the missed feed. This is, however, clearly at odds with the assumption drawn from the increase in abnormal and inactive behaviours with time following the false signal, which concluded that delays should be kept as short as possible.

The correlations found between rates of behaviours all thought to be indicators of anxiety in this species, and increasing time following the false signal, are therefore contradictory. Conversely, there was no significant difference with increasing time after the non-delivery of the expected food, on rates of self-scratching, which showed a significant main effect of signal condition. It is very difficult to explain these apparent anomalies. The fact that scratching, a behaviour widely thought to be an indicator of anxiety in primates in general (Maestripieri *et al*, 1992) was not affected, however, suggests that caution is necessary in drawing any conclusions at all from the results. Both behavioural (Bertrand, 1969) and physiological / pharmacological (Redmond & Huang, 1979) studies have found scratching to be associated with anxiety in this species in particular, suggesting that it is a robust indicator of anxiety in the stump-tailed macaque. The lack of correlation between scratching and time following the false signal may be taken as evidence that anxiety levels do not change over this time. An alternative view is that anxiety levels are immediately significantly raised following a false signal, and therefore even short delays may constitute a considerable welfare problem. The problem remains of explaining the correlations between rates of the other

behaviours and time following the false signal. Further studies could investigate whether the increases and decreases in the behaviours seen can be replicated, and if they can, to explore further possible reasons for them.

It is surprising that scratching, locomotion and foraging were the only behaviours affected by predictability of feeding in this study, as so many other behaviours are thought to be welfare indicators in this species. This is especially true as a within-subjects design was used in the study, which is powerful in comparison with the between-subjects design, making any significant differences easier to detect (Howell, 1989). It is possible that extraneous variables that could not be controlled for, such as staff carrying out other routine tasks at similar times each day which could have given the monkeys cues to feeding, may have minimised behavioural changes.

The fact that data were collected as soon as the new signal was introduced in Condition C may have meant that, for some of the observations, the animals had not yet learned the connection between the new, reliable signal and feeding. In order to minimise this problem it may have been beneficial to have exposed the animals to the signal-feeding pairing several times before data collection began, so that this connection might have been established. Alternatively, a similar design could have been utilised as was used in the marmoset studies described in Chapters 5 and 6. Here, 'Trial Period' was used as a factor in the analyses, allowing investigation into the how behavioural frequencies changed over the course of the study, as learning took place. Unfortunately, neither of these methods were used in the present study, primarily due to time constraints.

It is possible that some of the behaviours that were not affected by predictability of feeding are not in fact valid indicators of anxiety in this species. Alternatively, some of them may only be activated by anxiety involved with a particular type of situation. For example, allogrooming is thought to play a role in

the development and maintenance of social bonds (Dunbar, 1991; Schino *et al*, 1988) as well as being a tension reduction mechanism (Nieuwenhuijsen & de Waal, 1992; Boccia *et al*, 1989; Schino *et al*, 1988). The social context of allogrooming may mean that it is increased as a result of anxiety following social disruption but not anxiety associated with a missed feed. Further study is necessary to validate the above behaviours as indicators of anxiety in this species, as well as to investigate whether they are context-specific.

It is unfortunate that data were not collected relating to food delivery in respect to signal occurrence in the 'unreliable signal' condition. I did not record whether or not these signals were reliable or unreliable on each day for this condition, or whether they occurred at all. It was consequently impossible to split the data into 'reliable', 'unreliable' and 'no' signal days. It was therefore feasible that rates of scratching were high only if the normal feeding-related signals were unreliable, and lower if the signals were reliable. However, it is also possible that rates of scratching were high over the whole data collection period just before the normal feeding time, as unreliable signals often occurred at this time. This second possibility would suggest that exposing animals to feeding-related signals that are occasionally unreliable results in them experiencing a general increase in anxiety before their normal feeding time. This would be true even on occasions when the relevant signals are reliable and when they do not occur. Further studies would be necessary to determine whether this was the case. Further studies could also look at numbers of signals and time periods involved; for example, how many reliable signals an animal needs to be exposed to before it 'forgets' that the signal may be unreliable and therefore becomes less anxious prior to feeding time.

Probably the most serious shortfall of this study, however, was not including a condition that included both the original, naturally occurring unreliable signal as well as the reliable, experimentally administered signal. This would have enabled me to assess whether the beneficial effects of the reliable signal would compensate

for detrimental effects of the unreliable one, possibly by enabling the animal to habituate to the unreliable signal. The artificially sounded, reliable signal might then act as a buffer, making the animals better able to cope with, and avoid the stressful consequences of, the naturally occurring, unreliable signal.

The incorporation of a new reliable signal into existing feeding routines, if found to be effective, would probably be the most easily administered and therefore useful application of this research in the real world. It is not practical in most situations, for example, to expect feeding-related signals to be completely removed, nor is it realistic to expect delays to be eliminated and therefore for these signals always to be reliable. It would be more feasible to incorporate a reliable signal into the feeding routines of keepers and caretakers; for example, they could reliably blow a whistle a minute before feeding a particular group of animals and sound a buzzer before feeding another group. This is therefore an important area to investigate in further studies.

Lack of data on signal reliability in the 'unreliable signal' condition also meant that it was not known whether feeding was later on the days that the signal was unreliable. It is possible that this was the case. For example, technicians may have started to feed the animals at around the same time each day, and thus the initial feeding-related signals may have occurred. An increased time-lag between the signal and feeding may have meant that, in addition to the signal being rendered unreliable, feeding itself was significantly later than on days when the signal was reliable. However, Waitt and Buchanan-Smith (2001) demonstrated for the same population of stump-tailed macaques that delays in feeding did not result in any significant changes in behaviour compared with occasions when feeding was carried out on time. Instead, behavioural changes seen before feeding at the normal time were prolonged when food was delivered late. Feeding was here defined as 'late' if it occurred past one Standard Deviation of the routine time (Waitt & Buchanan-

Smith, 2001). These findings suggest that the additional variable of delayed feeding, which could not be verified, is unlikely to have affected results in the present study.

Another area that has not been explored here yet might be crucial in the application of this research is the length of the time period between the signal and food delivery. Two or three minutes were used in this study for the reliable signal, whereas the marmoset study (Chapter 5) used a one-minute interim between the two. These time periods were chosen fairly arbitrarily, simply for the ease with which they fitted into the experimental design. It is possible, however, that there is an optimum time lag between signal and food delivery, which may differ with species, age and even between individuals. Studies have explicitly addressed the question of whether the temporal relationship between events affects learning about the relationship between them. For example, rats learned most about the association between a tone and shock when the onset of the tone preceded that of the shock by eight seconds (Mahoney & Ayres, 1976). When the tone preceded the shock by 150 seconds, the rats showed no evidence of having learned anything about their association. Further studies could ascertain whether there is a similar effect between the pairing of a signal with an appetitive event such as feeding, and perhaps recommend approximate time periods for each species, age group and so on, depending on the findings. It would also be useful to assess how long it takes for conditioning to occur in different species; in other words, how many pairings of signal and food delivery are necessary before the animal makes an association between them.

Another area that this study has been unable to address is the 'window' between signal and food delivery within which the animal may receive the food without the signal becoming unreliable. For example, it may be the case that a macaque receiving its food between one and three minutes after a signal experiences the benefits afforded by the reliable signal in this study. In contrast, it is possible that if the food is always between one and *five* minutes after the signal the signal is

effectively unreliable and the animal becomes anxious as a consequence of this. This would correspond to the 'unreliable signal' condition in the present study. The feeding related sounds in this condition were always, paradoxically, reliable in that feeding always followed them. The essential difference between this and the 'reliable signal' condition was that the 'window' between signal and feeding, rather than being a fixed two minutes, was more elastic – between two and 90 minutes. This demonstrably had a negative effect on welfare, and it would be useful to examine how variable this time lag had to be before welfare was affected. Keepers would have to stick to a less rigid routine if, for example, feeding within a five-minute window after a signal offered the same benefits as feeding exactly two minutes after the signal. A less rigid routine might also be more useful in that it would be easier to follow without the occurrence of potentially damaging timing delays.

7.4 SUMMARY AND CONCLUSIONS

The results of this study support those described in Chapter 5; food delivery preceded by an unreliable signal appears to result in anxiety and reduced welfare for this species. This study supports the hypothesis that these earlier findings may be replicated in a real-life situation with a different species, thus increasing the generalizability of the results. Removal of salient unreliable feeding-related signals resulted in a reduction of self-scratching, a possible indicator of stress for this species. However, the removal of feeding signals is unlikely to be feasible in most real-life husbandry situations. Incorporation of a new, reliable signal did not result in any changes in behaviour compared with when no obvious signal was present. The presence of a signal in itself was therefore not responsible for the initially high stress levels, which were assessed for the purposes of the study by rates of scratching. However, if this new signal was not followed by the expected feed,

stress returned to a level not significantly different from that associated with the original, unreliable signal. The most important question now is to ascertain whether a new reliable signal may buffer the animal against the effects of an existing, deleterious, signal. This would represent a simple, inexpensive and easily incorporated modification to the normal routine of captive primates, which may nevertheless improve their welfare.

Chapter 8

General Discussion

8.1 Improving the welfare of captive primates

Maintenance of adequate welfare of laboratory and other captive animals is important to scientists and the general public for ethical, scientific and financial reasons. In particular, the importance of adequate laboratory animal welfare in order to ensure good quality science cannot be overemphasised. Scientific method assumes the absence of confounding variables; stress is such a potential confound (Poole, 1997). The environment in which experimental animals are housed tends to be standardised in order to minimise variability between study animals (van Zutphen *et al*, 2001). Scientists will generally assume that their experimental animals have normal blood pressure, heart rate, levels of stress hormones, immunological competence, digestion, heart rate and behaviour (Poole, 1997). However, the physical and social environments in which animals are kept, as well as in attitudes and handling by laboratory staff, have the potential to cause distress to animals and therefore affect their behaviour and physiology. For example, the standard barren laboratory cage is known to interfere with normal behavioural and brain development, affecting learning and memory (Cummings *et al*, 1977; Gardner *et al*, 1975; Renner & Rozeweig, 1987; Würbel, 2001), and represents a major cause of stress to laboratory animals (Ladewig, 2000). Paradoxically, it is likely that the standardised laboratory cage, designed to reduce variability between environments and hence study animals, is itself potentially a primary source of such confounds (Olsson *et al*, 2003).

Many studies have addressed issues involved in improving the environment for captive animals. A number of different ways to improve the environment and welfare of captive animals have been suggested; some of these will be described and

their shortcomings discussed. As primates are the focus of this thesis, work on this order will be emphasised.

It would appear to be intuitively obvious that increasing the amount of space available to animals would benefit their welfare. Reinhardt and Reinhardt (1999a) agree that a reasonable amount of space is necessary for captive primates in order for them to meet their basic needs for postural adjustment. However, they state that laboratory enclosures are traditionally unfurnished, and the space available to the animals is 'empty'. They claim that in addition to this empty space, primates also require 'functional space' for species-typical locomotion and interaction with a dynamic environment. Such functional space might include perches and swings, providing climbing opportunities and interaction with moving structures.

The minimum cage size for primates is regulated in Britain by the Animals (Scientific Procedures) Act (1986), and is directly related to the weight of the animals housed therein. However, increasing cage space is probably the most disputed improvement to the environment of laboratory primates, probably because it is relatively expensive (Bowden, 1988; Crockett, 1993; Crockett & Bowden, 1994; Hubrecht & Mason, 1993; Line *et al*, 1989b; Poole & Hubrecht, 1994). Increasing the size of primates' cages may be beneficial to their welfare provided that cage complexity and usable space are also increased (Reinhardt & Reinhardt, 1999b). If cage size cannot be enlarged, for example due to financial constraints, the complexity of the cage may still be increased with the provision of cage furnishings and manipulable objects.

Increasing cage complexity by introducing a woodchip floor covering has been found to increase activity and reduce aggression and abnormal behaviour in several species of primates (Chamove & Anderson, 1989). However, play and affiliative behaviour were also reduced in these animals, and these changes may be interpreted as being detrimental to welfare. The inclusion of novel objects has also been shown to reduce or eliminate abnormal behaviours of captive primates (Bayne

et al, 1993a; Bloom & Cook, 1989; Line & Morgan, 1991; Reinhardt, 1989). The use of objects (whether novel or familiar) in environmental enrichment for captive primates is generally considered to be a benign form of environmental challenge (Fragaszy & Adams-Curtis, 1991). The innocuous nature of the challenge is, however, dependent on the individual being able to control its interaction with the object. There is a danger that the type, quantity or duration of novelty may distress primates. Anxiety may result, for example, if a primate is unable to retreat a sufficient distance from the object, or if it is unable to respond appropriately (USDA, 1999). There are clearly implications of this for the provision of enrichment to primates housed in confined spaces.

The response of primates to the presence of novel objects introduced for enrichment purposes tends to be affected by habituation, the

> 'relatively permanent waning of a response as a result of repeated stimulation which is not followed by any kind of reinforcement. It is specific to the stimulation and relatively enduring'. (Thorpe, 1963, p. 61).

The fact that lack of reinforcement is necessary for habituation to occur explains why habituation is far less likely to happen with foraging-related enrichment devices, such as food puzzles. Similarly, the point that responsive objects are habituated to less quickly than non-responsive ones (Markowitz & Line, 1989) is likely to be because the control afforded by these objects acts as reinforcement, prolonging their use. However, the use of food puzzles may not be ideal in many captive situations, where obesity is often a serious problem for captive primates (Bloomsmith & Lambert, 1985). Additionally, the provision of novel objects may be considered to be expensive, especially as continual removal, cleaning and recycling of objects from a large 'pool' may be necessary in order to avoid habituation. Provision of objects, especially destructible ones, may increase the amount of time necessary for cleaning enclosures, an important consideration when staff time is restricted, as it often is in a busy facility.

Time constraints may also limit the ability of staff to provide different types of feeding enrichment such as food puzzles or foraging boards. An easier method of providing feeding enrichment is to provide food items such as fruit and nuts, whole rather than chopped, peeled or shelled. This may stimulate food processing behaviour and increase the time spent feeding (Nadler *et al*, 1992; Reinhardt & Roberts, 1997; Smith *et al*, 1989). However, care has to be taken to ensure that the animals do have or develop such skills, and therefore do not go hungry. Additionally, provision of whole food items may result in dominant animals more easily collecting and hoarding food, while subordinates are prevented from accessing desired items. However, scattering chopped food around the enclosure is easy, and may increase time spent foraging. It may also decrease aggression, as food in concentrated patches may be monopolised by dominant individuals, with agonistic behaviour increasing in squabbles over it (Sugiyama & Ohsawa, 1989). The standard ration may also be thrown on to the roof of cages in order to increase time-consuming foraging activities (Britt, 1993; Buchanan-Smith, 1995; Reinhardt, 1992b).

Social housing is generally believed to provide a very complex set of stimuli and hence be more enriching than any inanimate artefact (Visalberghi & Anderson, 1993; Novak & Suomi, 1988; Poole, 1991; Harris, 1988). Isolated animals frequently have impaired immunological function, believed to be a result of isolation stress, a phenomenon which is well documented in rodents (Baer, 1998). However, social housing may be associated with aggression leading to injury and stress (Reinhardt 1990b). Subordinate animals may be denied access to commodities, such as food, water, cover and visual barriers, and may be subject to social distress (Line *et al*, 1990; Novak & Suomi, 1988). Certain individuals, usually those of low rank, may become permanent scapegoats and experience persistent attacks from cagemates (Visalberghi & Anderson, 1993; de Waal, 1989). Aggression may occur at any time, and constant monitoring is necessary to ensure

the safety and welfare of the animals. Once again, this may cause problems if staff do not have the time to continually check their animals. However, where space, time, money and scientific protocol permit it, the benefits of social housing are generally thought to outweigh the risks involved (Baer, 1998; Reinhardt, 1990b; Visalberghi & Anderson, 1993).

The brief descriptions above have outlined various methods used to improve the environment of captive animals, together with some of their shortcomings. However, one aspect of the environment that has received little attention is the timing and predictability of husbandry routines. Although procedures such as feeding and cleaning are obviously essential to animals' physical health, their impact on psychological well being is rarely considered (Waitt & Buchanan-Smith, 2001). A free-living vertebrate will experience a degree of variability in the temporal and spatial relationships that it encounters in everyday life (Wiepkema & Koolhaas, 1993). Organisms are adapted to such natural variation and are generally able to cope with such unstable conditions. It has been suggested that a degree of environmental instability or uncertainty is necessary for animals in order to optimise vigilance and avoid boredom (Wiepkema & Koolhaas, 1993).

However, in captivity, animals may experience such a decrease in the predictability or controllability of their environments that stress may occur, leading to reduced welfare. One example of such variation may be feeding delays resulting in the absence of food at an expected location or time. As long as the animal is able to solve the problem, by increasing foraging or exploration in order to find food, the event should fall within the adaptive range of the organism and welfare should not be adversely affected. However, captivity often affords little opportunity for animals to exert control over relevant events in their environment (Ödberg, 1987; Wiepkema, 1987). Foraging may be impossible, or at least superfluous, with a lack of appropriate consequences. This may result in the motivation to forage never

becoming satisfied, according to the concept of behavioural needs (Hughes & Duncan, 1988; see Chapter 1 of this thesis). If this situation is prolonged the animal may become locked into a closed feedback loop in which the behaviour becomes increasingly stereotyped. The animal may be unable to cope, with its welfare seriously compromised (Wiepkema & Koolhaas, 1993).

Practical and financial considerations mean that it is often impossible to provide captive animals with control over their environment. Lighting and heating regimes are imposed, particular types and quantities of food are delivered, often at set times, and social groupings are determined by humans. Captive animals have little immediate control over resources that may be important to them, compared with animals in the wild which have a greater opportunity to interact with their environment (Taylor *et al*, 2001).

Even where opportunities for the control of appetitive events such as feeding have been provided, many of the devices used, and the stimuli provided for the animals to control, have been criticised as being very unnatural (Buchanan-Smith, 1997). Markowitz and Line (1989) showed that rhesus monkeys will touch a control switch several thousand times in order to receive a food reward. One individual did so 130 000 times in a week. This obviously interferes with 'natural' behaviour patterns and the desirability of such behavioural change has been questioned (Buchanan-Smith, 1997). The control of music has also been given to primates as enrichment (Markowitz & Line, 1989; Novak & Drewson, 1989). However, it has been suggested that other individuals in the same environment as the individual with control (which may be a dominant animal, or may be singly housed in a colony room) will experience welfare problems if they have no provision for direct control over the stimulus themselves (Buchanan-Smith, 1997).

In the absence of control, the timing and predictability of routine husbandry procedures may affect how they are perceived by the animals involved (Waitt & Buchanan-Smith, 2001). Feeding is likely to be the most important occurrence in

the day of a captive animal (Carlstead, 1986), yet variations in its timing and predictability have been the subject of little research. Despite such manipulations being relatively simple and inexpensive to implement, they may represent real opportunities to improve the welfare of captive animals. The broad aim of this thesis was to explore the effects on the behaviour, and inferred welfare, in two species of captive primates, of variations in the timing and predictability of feeding. This was in order to formulate recommendations relating to optimal timing and signalling of feeding, which was intended to reduce stress associated with feeding and ultimately improve the welfare of captive primates.

8.2 Indicators of welfare in the common marmoset

The thesis also sought to identify behaviours related to stress in one of the study species, the common marmoset. Although the common marmoset is commonly used in behavioural and biomedical research (Boyd Group, 2002), few studies have attempted to identify behaviours associated with reduced welfare in this species. This was problematic, as the common marmoset's popularity as a laboratory animal made it an ideal study species for the experiments described here. In order to be able to assess welfare implications of manipulations of feeding predictability, it was first necessary to have some, preferably non-invasive and simple to use, index of welfare in the common marmoset.

Chapter 4 describes a study carried out in an attempt to validate the use of both behavioural indices and urinary cortisol as measures of stress and reduced welfare in the common marmoset. One group of animals was trained using positive reinforcement to provide urine samples for analysis. It was found that trained and untrained animals responded differently to a mild stressor, with trained marmosets appearing to be better able to cope with it. Human interaction has been considered to be enriching for some species of primates (Bayne *et al*, 1993b). One of the goals of environmental enrichment has been defined as enabling animals to cope with

challenges in a more normal way (Young, 1998). The positive human interaction experienced by the marmosets as a consequence of the training procedure appears to have fulfilled this criterion and therefore may be considered to have been enriching to them.

The study has resulted in an index of welfare for this species, which were used for two of the studies described in the thesis. It was found that decreases in inactive behaviour, and increases in locomotion, self-scratching and scent marking could be used as indicators of welfare in the common marmoset. These findings have broad implications for the assessment and subsequent improvement of welfare in this species, as the measures used are simple, non-invasive and easy to implement. They could, for example, be used by technicians to assess welfare implications of variations in husbandry and scientific procedures. However, careful interpretation and a prudent approach is considered necessary when using changes in rates of these behaviours as indications of reduced welfare, as such changes may be due to factors unrelated to stress.

8.3 Effects of predictability of feeding on the behaviour and welfare of captive primates

The main body of the thesis describes studies that utilised behavioural measures in an attempt to assess the effects on captive primates of variations in the predictability of feeding. Studies investigating the effects of variations in predictability of both aversive and appetitive events have often been criticised as being complicated, confusing and questionable in terms of experimental validity (Weinberg & Levine, 1980; see Chapter 2 for a full review). It is suggested in the thesis that discrepancies between the results of studies exploring the effects of predictability of feeding may arise from the confusion of two distinct, but overlapping, types of predictability. 'Temporal' predictability relates to whether an event occurs at a fixed or variable time; an event occurring at exactly the same time

on each day would be temporally predictable. 'Signalled' predictability relates to the reliability of a signal preceding the event; a unique signal always occurring the same interval of time before an event would render its signalled predictability as high. This thesis attempts to tease apart the different effects of these two distinct but overlapping types of predictability. A third type of predictability is also explored for the purposes of the thesis; this relates to the reinforcement contingency ratio of trials.

The studies described in Chapters 5 and 6 were intended to examine specifically the effects of differing signalled and temporal predictability on marmoset behaviour and welfare. For the experiment concerned with signalled predictability (Chapter 5), three experimental groups and one control were used. Experimental groups were exposed to either a reliable signal, an unreliable signal or no signal before food delivery. All animals received their food on a temporally unpredictable schedule.

The preparatory response hypothesis (Perkins, 1955, 1968; Lockard, 1963) states that animals prefer signalled over unsignalled stimuli because when events are signalled, organisms can make preparatory instrumental responses which either decrease the aversiveness of negatively perceived events, or increase the reinforcing nature of appetitive events. Preference tests were not used in the study described in Chapter 5 and it was therefore impossible to ascertain whether the monkeys 'preferred' the reliable signal over either an unreliable one or no signal at all. However, the greatest frequency of stress-related behaviour occurred in the unreliable signal condition and the least in the reliable signal condition, with an intermediate level seen when no signal was used. These results imply that receiving food on an unpredictable temporal schedule may be a stressful experience for marmosets, and that this stress is attenuated by the presence of a reliable signal but magnified by an unreliable signal.

This contrasts with the findings of Bloomsmith and Lambert (1995) who found that feeding chimpanzees on an unpredictable temporal schedule, with no experimentally introduced signal, resulted in an increase in species-appropriate behaviour, which was interpreted as showing improved welfare. The difference in the results of that study and the present one could be attributed to the fact that the chimpanzees in the Bloomsmith and Lambert (1995) study *did* have access to feeding-related signals, despite these not being addressed specifically as variables in the study. These signals consisted of food vocalisations sounded by the few individuals that could see the keepers bringing the food out to be delivered. The signals may have been reliable enough to reduce the stress associated with feeding on an unpredictable temporal schedule, making the Bloomsmith and Lambert (1995) study equivalent to the reliable signal condition in the one described in Chapter 5 of this thesis.

The results of the Chapter 5 study, however, concur with findings of Carlstead (1986), who found that unreliable signals preceding temporally unpredictable food delivery were associated with increased frustration and aggression in pigs. Although increased agonistic behaviour was not seen in the marmosets in the study described here, the increases in stress-related behaviours that were observed are likely to have been indicative of increased frustration. There are several possible reasons why increased aggression was not seen. The animals were housed in well-established pairs, with food provided to the individuals in separate areas wherever possible. In contrast, Carlstead's pigs were housed in groups of eight, had been housed together for only two weeks when the study began, and had only four feeding places available to them. Additionally, the pigs were maintained on a semi-restricted diet, which may have been responsible for the increase in feeding competition and aggression that was seen.

It is possible that the presence of the signal allows for preparatory salivation to occur. Food delivery in the presence of anticipatory salivation is thought to be

more reinforcing than food in the absence of such salivation (Perkins, 1955), and may play a part in reducing stress-related behaviours associated with receiving the food on an unpredictable temporal schedule. The presence of an unreliable signal may be associated with salivation in the absence of food (when the signal is sounded but not followed by food delivery) which is thought to be more aversive than no salivation when there is no food (Perkins, 1955). The aversiveness of this salivation in the absence of food may be responsible for the increased stress-related behaviours seen in the unreliable signal condition.

The results of the Chapter 5 study, however, also support the 'safety-signal' hypothesis (Abbott *et al*, 1984), which states that in schedules with signalled predictability animals learn to predict periods of 'danger', identified by the presence of the signal, from periods of 'safety', identified by the absence of the signal. Periods of 'danger' are associated with a relatively steady level of threat and a high state of physiological arousal. In contrast, predictable conditions result in alternating states of arousal and relaxation, according to whether the signal is present or absent. Although this hypothesis was originally formulated to explain the preference of animals for signalled over unsignalled aversive stimuli, it may also be applied to situations involving appetitive stimuli, such as food. A reliable signal allows the animal to predict food delivery, while the absence of the signal reliably indicates to the animal that feeding will not occur. However, when the signal is unreliable, the animal responds to it whether or not it is followed by feeding. This is likely to result in a primary frustration reaction (Amsel, 1958) a motivational state that is by definition aversive.

If the safety-signal hypothesis is applied to the study presented in Chapter 5, the reliable signal allowed the animals to predict the occurrence of an intensely stimulating external event – feeding. In the absence of this signal, the arousal associated with the expectation of food would be reduced as the animal had reliable information that feeding would not occur. Animals fed on the same unpredictable

temporal schedule but without the presence of a signal were unable to differentiate the periods when feeding was imminent from periods when feeding definitely would not occur, as potential discriminative signals were absent. Therefore, they would have maintained a relatively high level of arousal, and subsequently a moderate level of stress, throughout the whole observation period. Animals exposed to an unreliable signal, however, would have responded to this signal, and when it was not followed by food, are likely to have experienced frustration.

Carlstead (1986) proposed that pigs exposed to an unreliable feeding signal treated unrelated stimuli in the external environment as feeding cues. She stated that the failure of these 'signals' to be followed by feeding led to increased frustration and aggression. It is possible that animals in the unreliable signal condition in the present study may also have perceived irrelevant environmental stimuli as feeding signals, which may have led to frustration at times during the trial period other than directly following the experimentally manipulated unreliable signal.

The 'safety-signal' hypothesis has been put forward to explain why, in short-term studies, unpredictable shock is associated with high levels of gastric ulceration, whereas in long-term studies, the reverse is true (Abbott *et al*, 1984). Long-term exposure to unpredictable shock is thought to result in adaptation, resulting in decreased arousal. However, this would not occur if the shock were predictable, as the arousal would be phasic, only occurring when the signal was present. It is possible that the same phenomenon might be seen if the present study had gone on for a longer period of time. If it had, more stress-related behaviour would have been expected when food was delivered on a temporally unpredictable schedule preceded by a reliable signal, as opposed to on a similarly temporally unpredictable schedule with no signal. However, it was not possible to extend the study, and it is therefore unknown whether adaptation would occur in relation to unpredictable feeding as it appears to in relation to aversive stimuli such as shock. Additionally, it is not

possible to ascertain whether the presence of an unreliable signal might interfere with this process, which has only been reported when a signal is absent, rather than when it is present but unreliable.

It is known that animals are capable of learning to anticipate regular feeding times, even when held in environments with either no, or irregular, diurnal deviations in light intensity to act as cues indicating day length (Johannesson & Ladewig, 2000). Most animals easily establish rhythms associated with anticipation of feeding on a predictable temporal schedule (Mistleberger, 1994), either by the entrainment of circadian rhythms or by periodic feeding (Boulos *et al*, 1980). These 'endogenous clocks' enable them to anticipate food delivery when it occurs on a temporally predictable schedule in captivity (Mistleberger, 1994). The study presented in Chapter 6 concentrated on temporal predictability of feeding with animals receiving food on either a fixed or variable time schedule, and using a similar experimental set up to that in Chapter 5.

The study in Chapter 6 also introduced a variable that had not been included in Chapter 5, although it may have been an important factor in the results. This was the reinforcement ratio of the trials. Animals in the unreliable signal condition in Chapter 5 received food on only 50% of trials, whereas those in the other two experimental conditions received it on every trial. It is possible that the increase in proposed stress-related behaviours seen in this condition may have been due to this factor, rather than to the presence of the unreliable signal.

In Chapter 6, then, trials were balanced in terms of this variable, with four experimental groups, and one control. One group in each temporal condition (fixed or variable) received food on either a 50% or 100% reinforcement schedule. Signals were not used in this study in any condition. However, my presence in front of the cage may have been a reliable signal (in conditions with a 100% non-contingent

reinforcement schedule) or an unreliable signal (in conditions with a 50% reinforcement schedule) that food would be delivered.

The least stressful (or, possibly, the most pleasant) experimental condition was thought to be the one in which food was provided on a predictable temporal schedule on all trials. In this condition the only significant change in behaviour compared to the control was a decrease in inactivity. Food delivered on all trials on an unpredictable temporal schedule was thought to be slightly more stressful, as there was significantly less inactivity and more locomotion in this condition compared to the control. However, both of these conditions could be regarded as incorporating a reliable signal, in my arrival in front of the cage. I always began the sessions as soon as I had arrived in front of the cage, and did not watch the animals at other times. A more elastic 'window' was present between the CS (my arrival in front of the cage) and the US (food delivery) in the condition in which food was given on an unpredictable temporal schedule than when it was given on a temporally predictable schedule. This illustrates the overlapping nature of the two types of predictability (signalled and temporal) recognised in the thesis. Temporal predictability could be conceived as predictability in relation to a signal. Examples of such a signal might include variation in artificial light intensity, the arrival of staff at a facility, staff carrying out unrelated but temporally predictable routines, or the arrival of an experimenter in front of the cage.

In the study described in Chapter 6, food provided on 50% of trials and on a predictable temporal schedule was thought to be the most stressful condition, as interactions revealed the lowest rates of inactivity and the highest rates of locomotion, scratching and scent marking to occur in this condition. Providing food on 50% of trials on an unpredictable temporal schedule was thought to result in stress levels intermediate between this and the temporally unpredictable condition with food delivered on all trials. Interactions between the variables also showed that stress levels rose throughout the study period for animals in the two groups

receiving food on a 50% reinforcement schedule. This suggests that this kind of schedule became cumulatively more stressful as time and trials went on, possibly as the association between my presence (an unreliable signal) and the food delivery became more established.

It appears from this study that in order to minimise stress and optimise welfare, captive marmosets should be fed on a predictable temporal schedule. However, unavoidable changes to a predictable schedule, such as occur through delays to the normal routine, are thought to be extremely stressful for animals (Waitt *et al*, 2001). Loss of predictability in a previously temporally predictable routine is thought to be detrimental to welfare compared to a schedule that has always been temporally unpredictable (Mineka & Kihlstrom, 1978). In order to explore this aspect of the problem, in a second stage of the study described in Chapter 6, further trials were carried out for each condition without giving any food. This was intended to represent a situation where food was delayed.

Interactions between the variables showed that animals that had previously received food on a temporally predictable schedule, and on a 100% reinforcement ratio, were strongly affected by this change with stress-related behaviours increasing when food was withheld. Similar changes were not seen in the other conditions. This result concurs with those shown by Shenger-Krestovnikova (described by Pavlov, 1927), Levine and colleagues (1972), Johannesson and Ladewig (2000) and Waitt and colleagues (2001), in which loss of predictability of reinforcement was associated with behavioural and physiological changes thought to be indicative of frustration. The changes seen in the present study were interpreted as showing that, although a temporally predictable routine may result in the lowest levels of stress for this species, deviations from the rigidity imposed by the routine may be extremely stressful and hence detrimental to welfare. A degree of unpredictability, be it temporal or related to the ratio of reinforcement, appears to 'buffer' animals against the harmful effects of a disruption to the normal routine.

It is suggested in the thesis, based on the results from the experiments in Chapters 5 and 6, that although a temporally predictable feeding schedule appears to be the least stressful delays to this may be detrimental to welfare. Therefore, on a practical level, the most beneficial schedule for feeding this species is a temporally unpredictable one. The unpredictable temporal nature of the schedule should offer a degree of protection against feeding delays, which may be inevitable in a busy facility. Additionally, without fixed, temporally predictable feeding times, animals do not show feeding anticipatory behaviour (FAA) (Mistleberger, 1994). Feeding on an unpredictable temporal schedule may include decreased agonism, self-directed and abnormal behaviours, inactivity and coprophagy before meals, suggesting improved welfare (Bloomsmith & Lambert, 1995; de Waal & Hoekstra, 1980; Waitt & Buchanan-Smith, 2001; Wasserman & Cruikshank, 1993). The inclusion of a reliable cue to signal food delivery would minimise the stress associated with this temporally unpredictable routine in itself. The signal should be unique, and presented a fixed time period prior to feeding.

It is important to realise that stress appears to occur in relation to the predictability of delivery of a tiny titbit. This is not a major feeding event and the animals were unlikely to be very hungry when they received the food. This indicates that variations in predictability of feeding have the potential to cause a very powerful effect on welfare.

The two studies that led me to these conclusions were, however, very artificial in their nature; the time periods involved were very short and the foodstuff provided was not the main meal of the day. It is therefore questionable whether the conclusions drawn would be valid in a real-life situation. In an attempt to circumvent this problem, I carried out a further study, described in Chapter 7. Here, I worked the experimental conditions around existing husbandry routines. I also used a different species as my study animals. An Old World monkey, the stump-

tailed macaque was chosen, as it was taxonomically very different from the New World common marmoset. It was proposed that any consistent results found between two such taxonomically distinct species would increase the generalizability of any consistent results obtained across other primate species. It was not considered necessary to carry out a preliminary investigation to identify stress-related behaviours in this species, as it has been fairly extensively studied (*e.g.* Bertrand, 1969; Fooden *et al*, 1985; Goosen, 1974a; Waitt & Buchanan-Smith, 2001). Behaviours thought to be associated with anxiety and stress in this species include self-scratching, yawning, autogrooming and abnormal behaviours such as eye poking, pacing, rocking and self-aggression (Bertrand, 1969; Waitt & Buchanan-Smith, 2001).

In the study presented in Chapter 7, experimental conditions were worked around existing feeding routines. Behavioural observations were initially made around feeding time without any experimental manipulation, in the presence of naturally occurring, unreliable signals, such as the sound of a bucket being filled. Following this, these signals were removed as far as was practicable, and in a third stage, an artificial, reliable signal was introduced. This was rendered unreliable in the final stage of the study.

In common with the results of the studies in Chapters 5 and 6, the findings of this study indicated that an unreliable signal preceding food delivery may result in stress and reduced welfare in the stump-tailed macaque. However, the results of the final study must be viewed with caution, as significant differences in scratching seen between the stages of the study were not present when significance levels were adjusted to accommodate post-hoc testing. However, the fact that similar results were found in a more applied and less tightly controlled setting improves confidence in the findings of the marmoset studies (Chapters 5 and 6).

Rates of scratching were high in the initial condition, when the naturally occurring, unreliable signal was present. This condition was not subject to

experimental manipulation and is likely to have been largely unchanged over a long period of time. This indicates that adaptation to the arousal induced by unpredictable feeding does not occur over time, as would be expected by the safety-signal hypothesis (Abbott *et al*, 1984). However, it remains unclear whether this is because the stimulus in question, feeding, is an appetitive one, and this adaptation has previously only been demonstrated in relation to aversive stimuli. The presence of unreliable signals could also be responsible for the lack of adaptation to unpredictable feeding when the unpredictability is signalled rather than temporal.

8.4 Limitations, improvements and further research

The main focus of the thesis involved exploration of the effects of predictability of feeding on the welfare of captive primates. In order to assess welfare implications of variations in predictability, behavioural indicators were used. Indicators of welfare had not been identified for the common marmoset, and the first study of the thesis attempted to address this issue. However, the results of this study did not lead to unequivocal conclusions, possibly because of some of the methods used.

The facilities at the MRC unit were excellent, and enabled access to a large number of monkeys for use in the experiments. However, despite a reasonable sample size, administration of a stressor produced no discernible effect on urinary cortisol. Because of this, it was not considered meaningful to carry out correlations between urinary cortisol concentrations and rates of different behaviours, as had been intended initially. This is in contrast to studies that have reported increases in urinary cortisol following a stressor in Weid's black tufted-eared marmosets and cotton-top tamarins, both callitrichid species (Smith & French, 1997; Ziegler *et al*, 1995). Various methodological problems that may have contributed to the lack of significant results in the present study are described in the relevant chapter.

However, a more fundamental problem may have contributed to the lack of significance of these results.

The nature of the stressor, and the situation in which it is applied, will affect the stress indices activated. For example, Mendl and co-workers (1992) found that long-term social stress resulted in increases in cortisol in pigs, yet confinement showed less clear-cut effects. It has been suggested (Henry & Stevens, 1977) that the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for the secretion of glucocorticoids, may act separately from the sympathetic adrenomedullary system, which secretes catecholamines, in response to stressful stimuli. According to this proposal, the HPA system is activated by an individual's lack or loss of control over a situation, and results in behaviours associated with withdrawal, depression and subordination. In contrast, the adrenomedullary system results from the attempts of an individual to cope actively with a situation or stressor, and is behaviourally characterised by arousal and aggression.

Manipulations demanding active coping and control include shock avoidance (Schneiderman, 1983). Unpredictable environmental conditions, including temporal availability of food, may result in a perception of uncertainty, which triggers the HPA axis and results in increased glucocorticoids (Reneerkens *et al*, 2002). Elevated glucocorticoids may result in increased exploration (McIntyre, 1976; Veldhuis *et al*, 1982) and improved spatial memory (Luine *et al*, 1996), both useful adaptations when food supplies are unpredictably variable. It is possible that the nature of the stressor was such that the HPA axis was not activated, as the animals were able to employ an active coping strategy during its administration. However, it is difficult to imagine how they could have done this when faced with human handling and isolation in a novel cage, manipulations over which the animals had no control.

The stressor used in this study had a fairly short time span (between four minutes and 4 minutes 30 seconds). Smith and French (1997) used isolation in a

novel cage for eleven hours as a stressor; the longer time period of this stressor may explain the difference between these results and the ones obtained in the present study. In contrast to this, handling rats for just 30 seconds was enough to double plasma glucocorticoid levels in rats (Kvetansky *et al*, 1978). However, different species may show widely divergent physiological and behavioural responses to the same stressor (Mason & Mendl, 1993).

It is, however, possible that it was the severity of the stressor, rather than its nature, which was insufficient to result in a significant increase in cortisol in the present study. The severity of the stressor may have been further reduced by the fact that it was mainly carried out by a handler that the marmosets were familiar with, and had trained them using positive reinforcement. Positive human interaction is known to improve the relationship between primates and humans, consequently reducing the animals' fear of these individuals (Heath, 1989). It is possible that, had the people handling the marmosets been unknown to them, the stressor may have been perceived as more severe, resulting in significant increases in urinary cortisol.

It is also possible that measurement of cortisol concentrations may simply not be a useful index of stress for this species. Contrary to expectations, common marmosets of five to nine years old, which had never been outside before, did not show an increase in salivary cortisol on release into outdoor cages (Pines *et al*, 2002). Measurement of the catecholamines adrenaline (epinephrine) and noradrenaline (norepinephrine) may be useful in assessing the impact of acute stressors (Broom & Johnson, 1993). These hormones are secreted from the adrenal medulla within one or two seconds of the stimulus that initiated their release. However, they are notoriously difficult to measure, as they are very quickly metabolised, with a half-life in rat blood of just 70 seconds (McCarty, 1983). Levels of catecholamines in urine are of limited use, as they are extremely variable (Baum *et al*, 1982). Blood sampling must be carried out rapidly (using an

intravascular cannula) to be of any use (Broom & Johnson, 1993). However, this method is fairly invasive and may itself be responsible for activation of stress indices, particularly in nervous animals such as the common marmoset. Restraint is also likely be necessary for the method to be feasible. Measurement of blood pressure and heart rate, both of which may be a useful measure of the emotional response of an individual to short-term problems may be carried out remotely through telemetry, although this initially involves invasive surgery (Broom & Johnson, 1993; Gerber *et al*, 2002). Further studies investigating the effects of stressors on hormonal (*e.g.* testosterone, adrenaline and noradrenaline) or physical (*e.g.* heart rate and blood pressure) stress indices would be useful in order to further validate the behaviours described in this thesis as welfare indicators. Additionally, it would be extremely useful to examine the effects of different kinds of stressor (*e.g.* social stressors, isolation and human handling) on the behaviour and physiology of the common marmoset.

The stressor imposed in the study described in Chapter 4 consisted of isolation and human handling. This was different to the potential stressors under investigation in the later studies, which involved differences in the predictability of food delivery. However, the assessment of stress in these studies depended on the behaviours identified in the first one. It is possible that the differing nature of the stressors may have resulted in different stress responses, and hence different behavioural changes. The predictability studies were intended to assess whether these manipulations were indeed stressful, whereas the initial study used stimuli that I had predicted *would* be stressful. It was necessary to begin with the value judgement that isolation and handling would be perceived as unpleasant by the marmoset. It was stated in the first chapter of this thesis that the assessment of animal welfare inevitably involves value judgements, and it is difficult to see how the study could have been conducted without such a presupposition being made.

The studies described in Chapters 5, 6 and 7 of the thesis show that differences in the predictability of an appetitive event, feeding, have the potential to significantly affect the welfare of captive primates. Attempts were made to tease out the effects of two types of predictability, signalled and temporal predictability. A further type of predictability was also identified for the purposes of the experiments in Chapters 5 and 6. This related to the reinforcement ratio of trials. The conditions present in the signalled predictability study (Chapter 5) and the temporal predictability study (Chapter 6) are shown in Tables 8.1 and 8.2.

Table 8.1 Conditions used in the signalled predictability study (Chapter 5)

Condition	Temporal predictability	Signal	Reinforcement ratio
A	Unpredictable	Reliable	100%
B	Unpredictable	None	100%
C	Unpredictable	Unreliable	50%
D	Control	Neither signal nor food	

Table 8.2 Conditions used in the temporal predictability study (Chapter 6)

Condition	Temporal predictability	Signal	Reinforcement ratio
A	Predictable	None	100%
B	Unpredictable	None	100%
C	Predictable	None	50%
D	Unpredictable	None	50%
E	Control	Neither signal nor food	

It can be seen from these tables that although many combinations of the three variables were used, some were omitted. Additionally, it was not possible to compare the data from the two studies, as there were several significant differences between the two sets of control data. In order to cover all the possible combinations of the variables, and therefore to be able to separate their effects more easily, it

would have perhaps been better to have carried out one study, with the conditions shown in Table 8.3.

Table 8.3 Proposed conditions for a further study to compare the effects of the three variables

Condition	Temporal predictability	Signal	Reinforcement ratio
A	Unpredictable	Reliable	100%
B	Unpredictable	Reliable	50%
C	Unpredictable	None	100%
D	Unpredictable	None	50%
E	Unpredictable	Unreliable	50%
F	Predictable	Reliable	100%
G	Predictable	Reliable	50%
H	Predictable	None	100%
I	Predictable	None	50%
J	Predictable	Unreliable	50%
K	Control	Neither signal nor food	

There are ten experimental conditions, and one control, in the proposed study. To remove order effects, a between-subjects design would be necessary. In order to preserve the reasonable sample sizes that were used in the studies described here, it would be necessary to use 66 pairs of marmosets. This is a very large number of animals, even for a large facility such as the MRC. This would also be an extremely time-consuming study to carry out, and would necessitate more than one experimenter if the inter-trial intervals were to be kept reasonably short (trials were generally between one and three days apart in the experiments in the thesis).

Additionally, it would be useful to include similar stages during this follow-on study as were used in the experiment presented in Chapter 6. Therefore, at least twelve trials could be carried out in each condition as indicated in Table 8.3. However, a further set of trials (perhaps four, as were used in the Chapter 6

experiment, which led to interesting results), where no food was given should be added as a second stage. This would enable examination of the effects of loss of both signalled and temporal predictability. It would also be useful to ascertain whether animals accustomed to receiving a signal before a piece of food would be less affected by the absence of the food if the signal was also omitted than those that continued to receive the signal, but not the food. If animals receiving neither food nor signal were less severely affected than those still receiving the signal but not the food, it would suggest a valuable function of a signal to protect against the detrimental effects of delays to feeding, should they unavoidably occur.

The presence of the experimenter in front of the cage could be thought of as a powerful signal preceding feeding, be it reliable or unreliable. In order to remove this potentially confounding cue to feeding from all the conditions, it would be interesting to carry out the study using automatic feeding apparatus and video, so that the experimenter could be absent or in a hide during trials. This would enable a more complete dissociation of the two types of predictability under investigation.

The strictly controlled conditions that were used for the marmoset studies would be useful for the exploration of a number of factors associated with the issue of predictability. For example, it would be useful to explore the effects of varying the time period between the signal, when used, and the food delivery. Fairly arbitrary time periods were used in the experiments in the thesis; one minute for the marmosets, and two or three minutes for the macaques. These were not manipulated in these studies. The amount of time taken both to establish and extinguish a learned association between the unconditioned stimulus (feeding) and the conditioned stimulus (the signal) would also be useful to know. Two signals, one reliable and one unreliable, could also be used, in order to discover the relative importance of each. This could be important if, for example, the unreliable signal was found to be more salient to the animal. This would mean that the idea of using

a reliable artificial signal to buffer against the effects of a naturally occurring but unreliable one would be unlikely to be successful.

Additionally, different types of signal could be investigated. Auditory signals are perhaps the most obvious, but visual stimuli might be just as effective. The sight of a technician walking past the window with a bucket, for example, appeared to be enough to elicit vocalisations from some of the stump-tailed macaques at the MRC. Visual signals would provide an added advantage in that they could be restricted to just one group, even when groups are housed in close proximity. In such a situation it may be impossible to deliver an auditory signal to one group without the sound being heard by others.

The study presented in Chapter 7 was designed to move the focus of the investigation from a very artificial, theoretical situation to a more realistic one. It was much harder to control the variables in this study, yet the results suggested that the presence of unreliable cues to feeding may be stressful to this species. The only behaviour significantly affected by the signal condition in this study was self-scratching. However, studies have found self-scratching to be associated with anxiety in primates in general (review by Maestripieri *et al*, 1992) and the stump-tailed macaque in particular (Bertrand, 1969). The significant effect on this behaviour of the different signal conditions may reasonably be assumed to represent differences in anxiety levels of the animals. Post-hoc *t*-tests showed more scratching occurring in the conditions with unreliable signals than in the conditions where the signal was either reliable or completely removed. This is in accordance with the marmoset studies, which also found unreliable signals to be stressful.

However, no post-hoc *t*-tests were significant once the Bonferroni correction had been applied. This is not to dismiss the importance of the results, however, as Bonferroni tests are thought by some to be excessively conservative (Everitt, 1996). Rather, the results must be viewed with caution, as must any conclusions drawn from them. It is possible that had the sample size been larger, these tests would

have remained significant despite the adjustment of significance levels. Additionally, it is possible that had the study period continued for longer than it did, the association between the signal and feed, and the reliability of the signal, might have been more established, and so significant results may have been obtained.

It has been argued (Piersma & Ramenofsky, 1998) that red knots *(Calidris canutus)* are slower to adjust to a temporally reliable feeding schedule when accustomed to a temporally unreliable one than vice versa. The authors suggest that once the animals experience a temporally constant feeding regime, they 'remember' for a considerable period of time that conditions may revert to being unpredictable. They therefore maintain elevated corticosteroid levels and behavioural adaptations associated with an unpredictable schedule. The present study involved the manipulation (*i.e.* removal and replacement) of existing signals, which the animals had been accustomed to hearing for a number of years. These signals were unreliable (and hence feeding fairly unpredictable), and it is possible that it may take animals longer to adjust to reliable signals (and so predictable feeding) when accustomed to unreliable ones than vice versa.

There is also a possibility that, although signals of varying reliability did have some effect on stress-related behaviours, the effect was simply not as great in the real world as in the artificially constructed representation used in the marmoset studies. Possible reasons for this include the presence of other cues that could not be controlled, such as the staff carrying out non-feeding related routines. Observations were made around the main feed of the day for these monkeys, so it is possible also that hunger was a cue.

One other difference between the marmoset and macaque studies that may explain the discrepant findings was the amount of attention they paid to me. The attention of the marmosets during these studies appeared to be fixed on me, and it was a fairly unusual occurrence for them to be observed for such long periods at a time. This may have made any signals that I did make especially salient to them.

By contrast, the macaques appeared to ignore my presence by the window, and to a large extent, also the artificial signals that I sounded. They may not have perceived these signals as particularly relevant.

The main drawback of the study on the macaques was that the effects of the newly introduced reliable signal were not tested in combination with the reintroduction of the naturally occurring unreliable signals. If it was found that a reliable signal could compensate for the detrimental effects of unreliable, unavoidable signals, the incorporation of a reliable signal could be an easily implemented potential benefit to welfare. This would perhaps be the most important finding of this thesis. Unfortunately, this remained untested, yet would be an obvious candidate for further research.

This thesis has presented findings that suggest that taxonomically distinct primates appear to be similarly affected by variations to the predictability of feeding. It would be useful to test the generalizability of the results to other primate taxa, such as apes and prosimians. Additionally, studies could be carried out on other mammalian orders, or even extended to include other vertebrate classes.

It would also be useful to see whether the findings presented in the thesis are generalizable to other positively perceived stimuli, other than feeding. Examples of such stimuli might include positive human interaction, being reunited with conspecifics, or having visual access to human activity outside the enclosure, which may be enriching to captive primates (Waitt *et al*, 1999). It would also be extremely valuable to assess the impact of predictability of aversive stimuli on welfare. Examples of aversive stimuli to which laboratory primates may be exposed include cage cleaning, collar cleaning, blood draws, separation from young and manual palpation for pregnancy detection. Delays to cage cleaning, which itself is thought to be at least mildly stressful (Line *et al*, 1991), are thought to be detrimental to the welfare of captive primates (Waitt *et al*, 1999). Manipulating the temporal predictability of aversive events, or introducing 'safety' signals, may be a

relatively simple and inexpensive way of reducing their impact on welfare. It is likely that procedures, such as manual palpation for pregnancy detection, which are carried out infrequently, would be less affected by predictability than ones carried out more frequently, such as cage cleaning. This is because if a procedure is carried out less often, animals will have fewer opportunities in which to learn to predict it.

If the effects of predictability of events differ according to whether animals find them positive or negative, it is possible that deliberate manipulation of predictability may serve as a tool to help us understand how animals perceive those events. This might serve as a useful adjunct to other methods, such as preference tests, which allow scientists an insight into how animals view the world (Dawkins, 1990). An objective understanding of animals' perceptions of husbandry and scientific manipulations may represent a step towards improving their welfare.

8.5 Recommendations relating to the timing and predictability of feeding routines for captive primates

In order to minimise the detrimental effects on welfare seen in captive primates around feeding time, this thesis has presented findings indicating that animals should be fed on a temporally unpredictable schedule. However, feeding should always be preceded by a reliable signal, presented a fixed time before food is delivered. If different groups are housed in close proximity, with the consequence that signals may be perceived by more than one group at a time, the signals should be unique to each group. This will allow flexibility for keepers to feed groups at different times, if necessary. Signals should also only occur prior to feeding, and not at any other time.

8.6 Final comment

The question that I set out to answer in this thesis was whether variations in the predictability of feeding might affect the behaviour and welfare of captive

primates. The studies presented here suggest that such variations have the potential to cause severe welfare problems. Conversely, an understanding of the effects of predictability and careful manipulation of feeding routines may result in real welfare improvements for captive primates. Moreover, these changes may be simple and inexpensive to implement. Despite the theoretical interest that the studies may raise, it should not be forgotten that the results should be applicable to real-life situations. Animal welfare is an applied science and it is important that the results of the studies in this thesis, as well as those that might be achieved through further research, should be utilised in order to improve the welfare of captive animals.

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Appendix 1

Appendix to Chapter 5 – Effects of reliability of feeding-related signals on the behaviour and welfare of common marmosets.

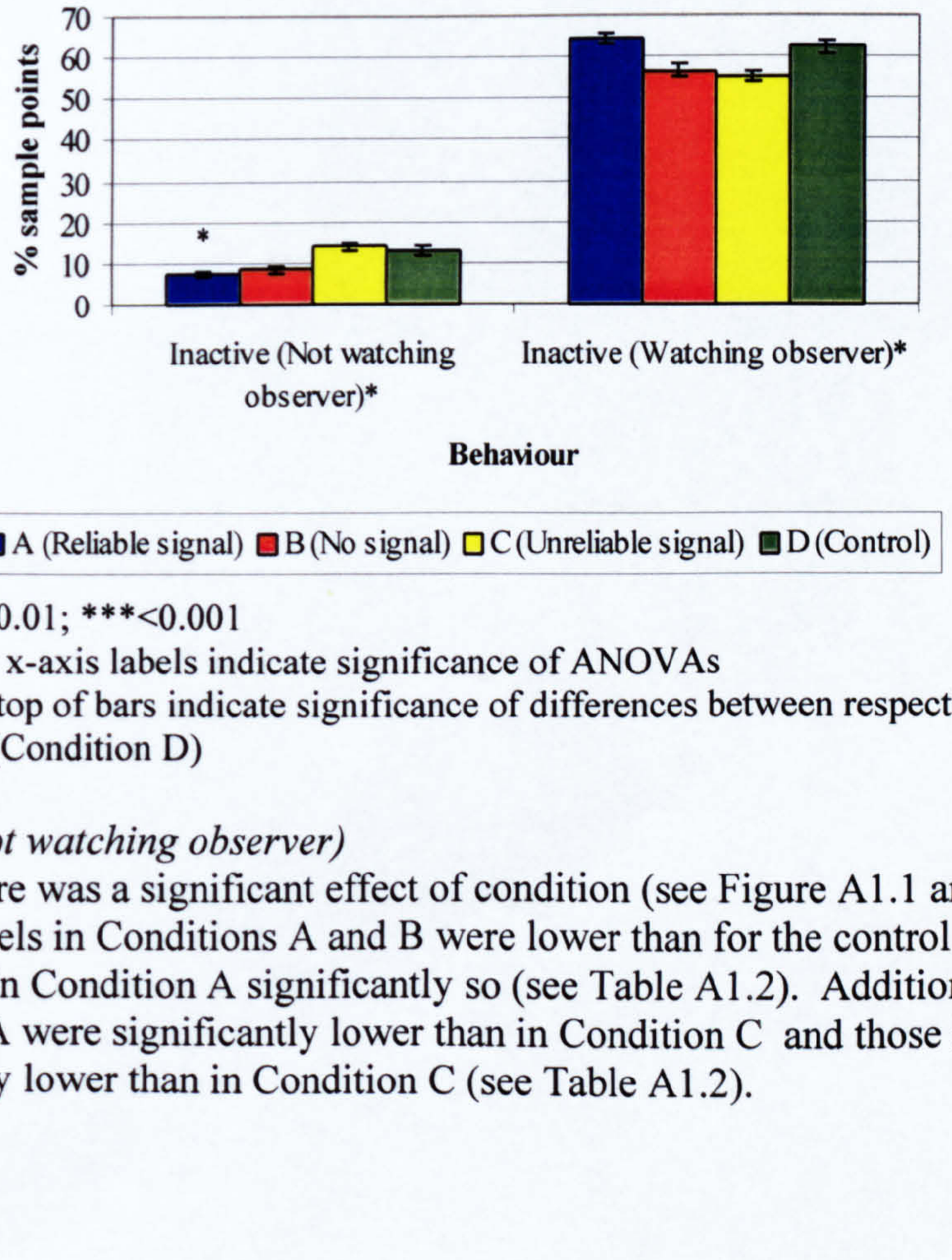
Chapter 5 described changes in various categories of behaviour, of variations in the reliability of a feeding-related signal. The behaviours described in that chapter were all thought to be relevant to welfare assessment. This appendix contains details the two sub-categories of inactivity – 'inactive, alert (not watching observer)' and 'inactive, alert (watching observer)', as well as 'vocalise', which were not included in the main chapter as their use as welfare indicators was not established in the common marmoset. However, each category was affected by signalled predictability, and the results and a brief discussion are hence presented here for the interested reader.

Effects of food deliveries of varying predictability on behaviour

The effects of each main factor ('Signalled Predictability', 'Trial Period' and 'Signal Period') are initially considered separately. Interactions between the variables follow these results.

Effects of Signalled Predictability of food delivery on behaviour

Figure A1.1 Percentage sample points spent inactive in the four study conditions (bars represent Standard Errors)



*<0.05; **<0.01; ***<0.001
Asterisks by x-axis labels indicate significance of ANOVAs
Asterisks at top of bars indicate significance of differences between respective condition and control (Condition D)

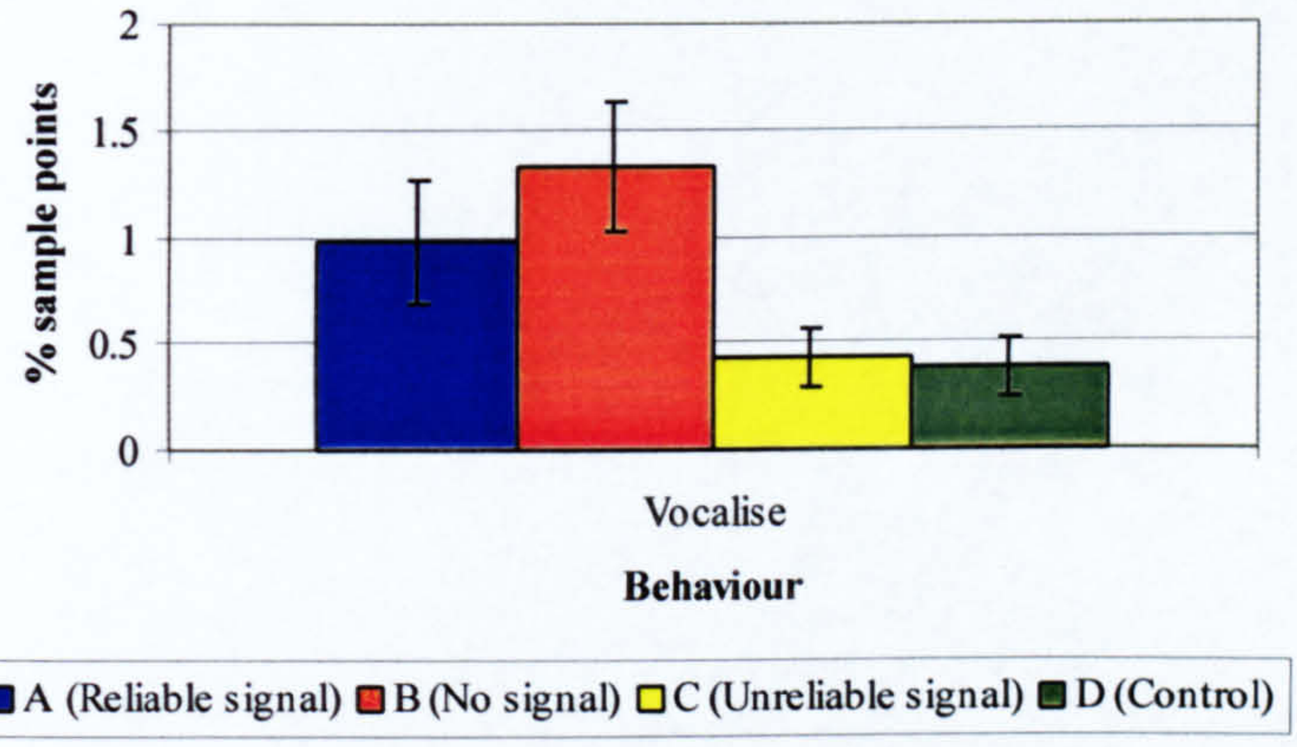
Inactive (not watching observer)

There was a significant effect of condition (see Figure A1.1 and Table A1.1). Levels in Conditions A and B were lower than for the control condition, but only those in Condition A significantly so (see Table A1.2). Additionally, levels in Condition A were significantly lower than in Condition C and those in Condition B significantly lower than in Condition C (see Table A1.2).

Inactive (watching observer)

There was a significant effect of condition (see Figure A1.1 and Table A1.1). Levels in Condition A were significantly higher than in Condition C (see Table A1.2). Mean levels of inactivity while watching the observer were lower for Conditions B and C than for the control, but these differences were not significant (see Table A1.2).

Figure A1.2 Percentage sample points spent vocalising in the four study conditions (bars represent Standard Errors)



Vocalising

There was no significant effect of condition on this behaviour (see Figure A1.2 and Table A1.1).

Table A1.1 Results of ANOVAs for effects of signalled predictability of food delivery on behaviour

Behaviour	F	p
Inactive (not watching observer)	7.70	<0.05*
Inactive (watching observer)	4.32	<0.05*
Vocalise	1.09	0.38

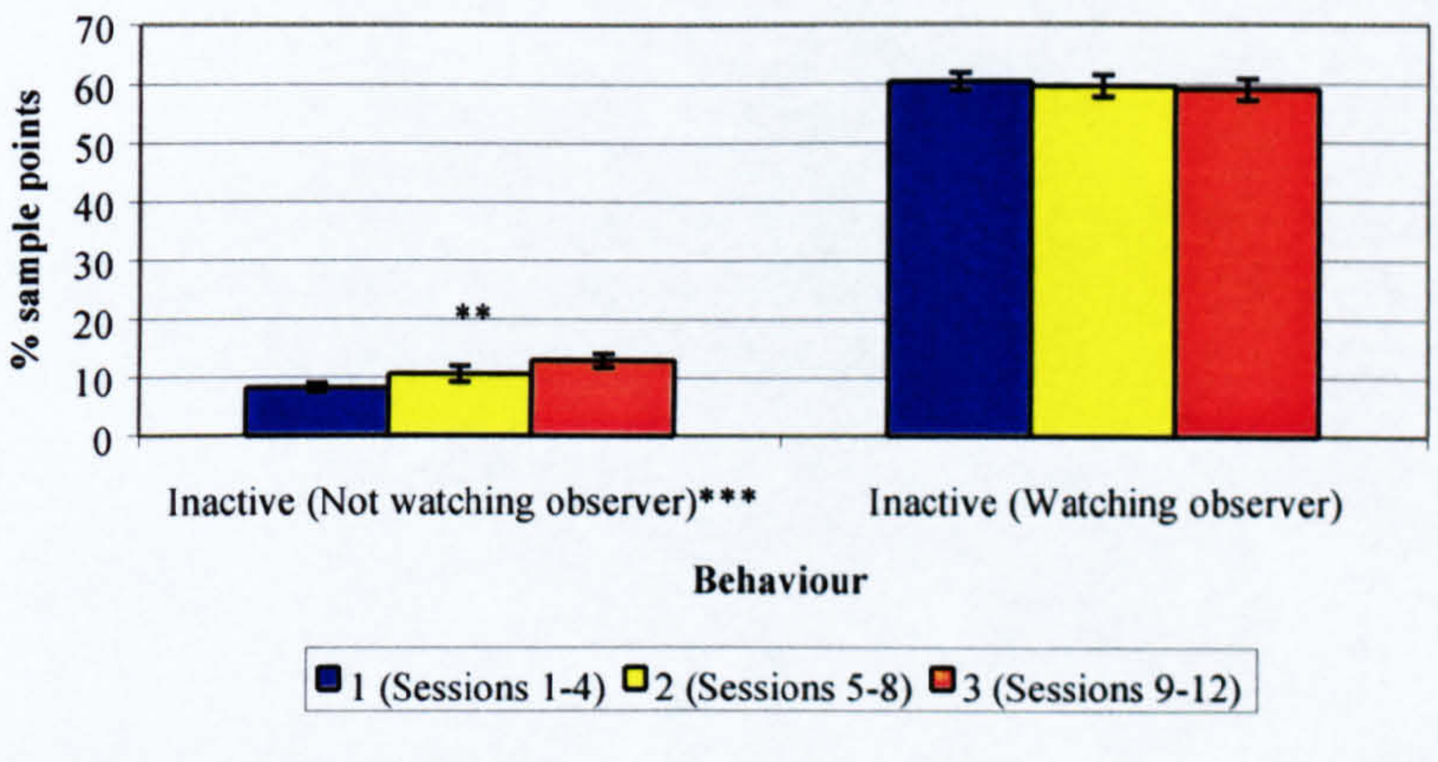
Table A1.2 Results of post-hoc Tukey tests for effects of signalled predictability of food delivery on behaviour (only behaviours showing a significant main effect of 'signalled predictability' are included)

Behaviour	Comparison between conditions	Mean Difference	S.E.	p
Inactive (not watching observer)	A-B	1.01	1.67	0.93
	A-C	6.63	1.67	<0.01**
	A-D	5.53	1.67	<0.05*
	B-C	5.61	1.67	<0.05*
	B-D	4.51	1.67	0.06
	C-D	1.10	1.67	0.91
Inactive (watching observer)	A-B	7.93	3.03	0.07
	A-C	9.32	3.03	<0.05*
	A-D	2.29	3.03	0.87
	B-C	1.39	3.03	0.97
	B-D	5.64	3.03	0.28
	C-D	7.03	3.03	0.13

Effects of trial period on behaviour

Each behavioural category is again presented separately. Where a significant main effect was found, results of linear trend tests are given.

Figure A1.3 Percentage sample points spent inactive in the three stages of the study (bars represent Standard Errors)



*<0.05; **<0.01; ***<0.001

Asterisks by x-axis labels indicate significance of ANOVAs

Asterisks above centre bars indicate significance of linear relationship between trial period and levels of respective behaviour

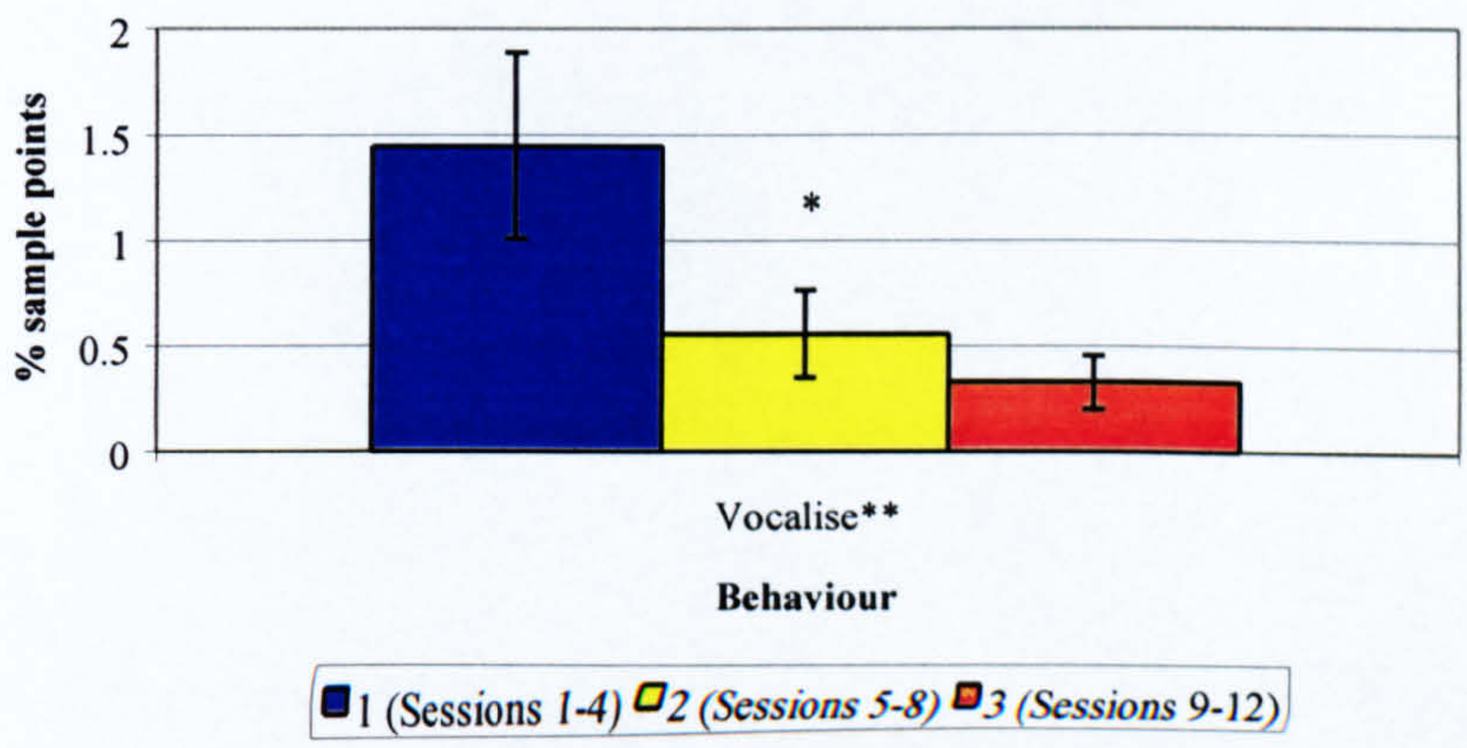
Inactive (not watching observer)

There was a significant effect of trial period (see Figure A1.3 and Table A1.3). There was a significant linear relationship between trial period and level of this behaviour; levels of inactivity while not watching the observer increased as trial periods progressed (see Table A1.4).

Inactive (watching observer)

There was no significant effect of trial on this behaviour (see Figure A1.3 and Table A1.3).

Figure A1.4 Percentage sample points spent vocalising in the three stages of the study (bars represent Standard Errors)



*<0.05; **<0.01; ***<0.001
Asterisks by x-axis labels indicate significance of ANOVAs
Asterisks above centre bars indicate significance of linear relationship between trial period and levels of respective behaviour

Vocalising

There was a significant effect of trial (see Figure A1.4 and Table A1.3). There was a significant linear relationship between trial period and level of this behaviour; levels of vocalising decreased as trials progressed (see Table A1.4).

Table A1.3 Results of ANOVAs for effects of 'Trial Period' on behaviour

Behaviour	**F**	**p**
Inactive (not watching observer)	10.66	<0.001***
Inactive (watching observer)	0.43	0.66
Vocalise	6.35	<0.01**

All d.f. = 2,40

Table A1.4 Results of linear trend tests for effects of 'Trial Period' on behaviour (only behaviours showing a significant main effect of Trial Period are included)

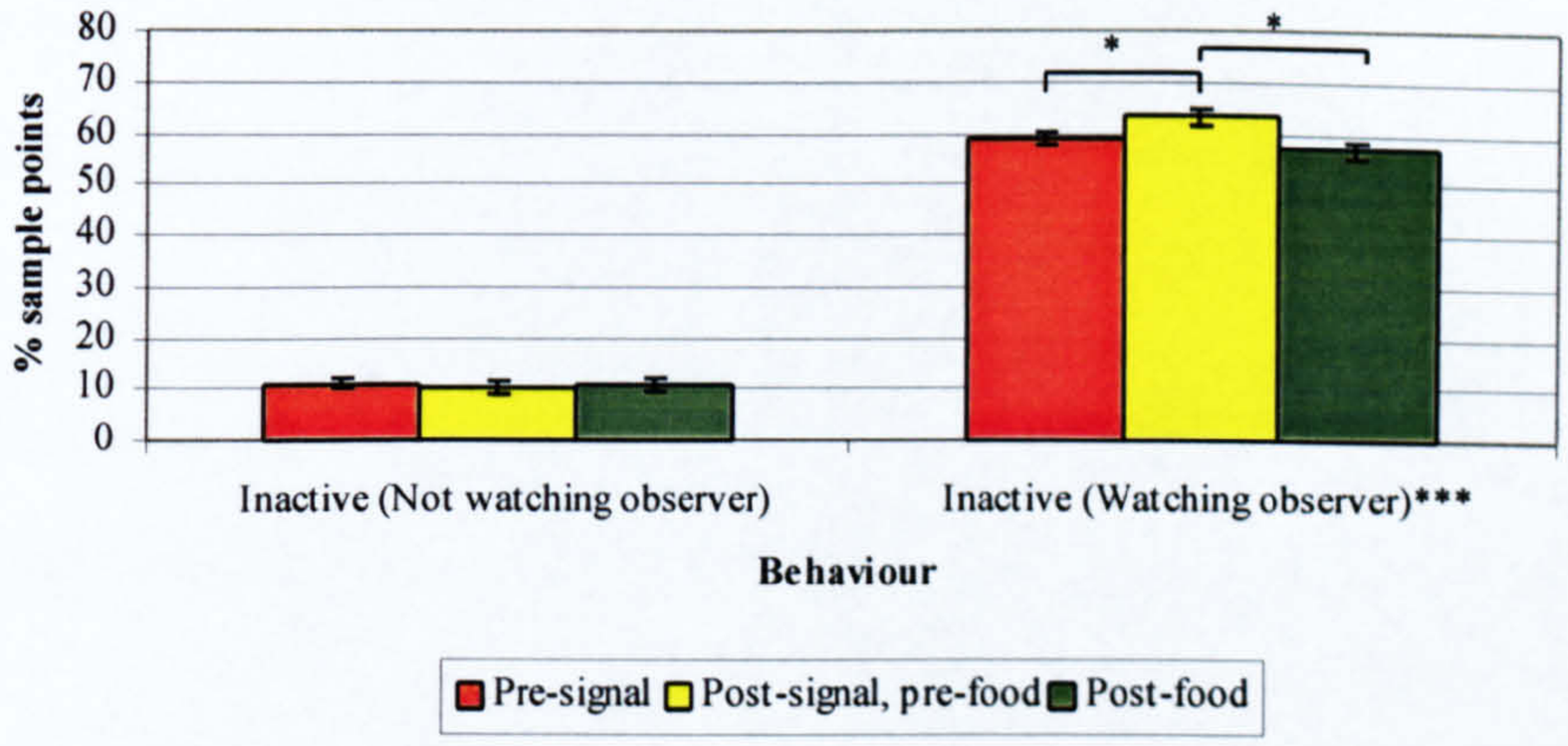
Behaviour	**F**	**p**
Inactive (not watching observer)	16.19	<0.01**
Vocalise	7.63	<0.05*

All d.f. = 1,20

Effects of signal period on behaviour

Each behaviour is again presented separately. Where a significant main effect was found, results of post-hoc pairwise *t*-tests (with the Bonferroni correction) are given.

Figure A1.5 - Percentage sample points spent inactive in relation to the time of signal presentation within the session (bars represent Standard Errors)



*<0.05; **<0.01; ***<0.001

Asterisks by x-axis labels indicate significance of ANOVAs

Asterisks above boxes indicate significance of post-hoc pairwise *t*-tests (following Bonferroni correction)

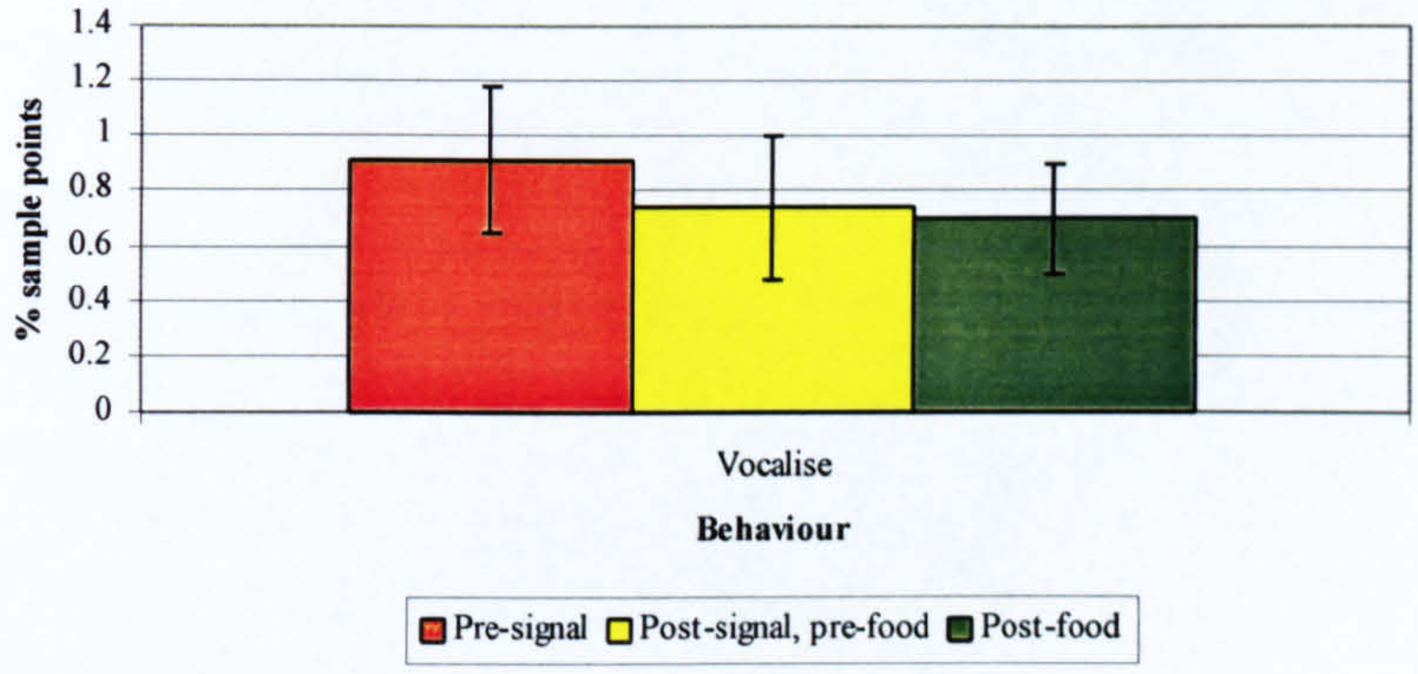
Inactive (not watching observer)

There was no significant effect of signal on this behaviour (see Figure A1.5 and Table A1.5).

Inactive (watching observer)

There was a significant effect of signal (see Figure A1.5 and Table A1.5). In a similar pattern to that found for the 'Inactive (all categories)' behavioural category, levels of inactivity while watching the observer were significantly lower in the pre-signal period than in the post-signal, pre-food period, and significantly higher in the post-signal, pre-food period than in the post-food period (see Table A1.6).

Figure A1.6 - Percentage sample points spent vocalising in relation to the time of signal presentation within the session (bars represent Standard Errors)



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Vocalising

There was no significant effect of signal (see Figure A1.6 and Table A1.5).

Table A1.5 Results of ANOVAs for effects of 'Signal Period' on behaviour

Behaviour	**F**	**p**
Inactive (not watching observer)	0.52	0.60
Inactive (watching observer)	10.88	<0.001***
Vocalise	0.70	0.50

All d.f. = 2,40

Table A1.6 Post-hoc *t*-test *t* and p values for mean percentage sample points spent in each behaviour in each 'Signal Period' (only behaviours showing a significant main effect of Signal Period are included)

Behaviour	**Signal period**	***t***	**p** (uncorrected)	**p** (following Bonferroni correction)
Inactive (watching observer)	1-2	2.93	<0.01**	<0.05*
	1-3	1.58	0.13	0.39
	2-3	3.77	<0.001***	<0.01**

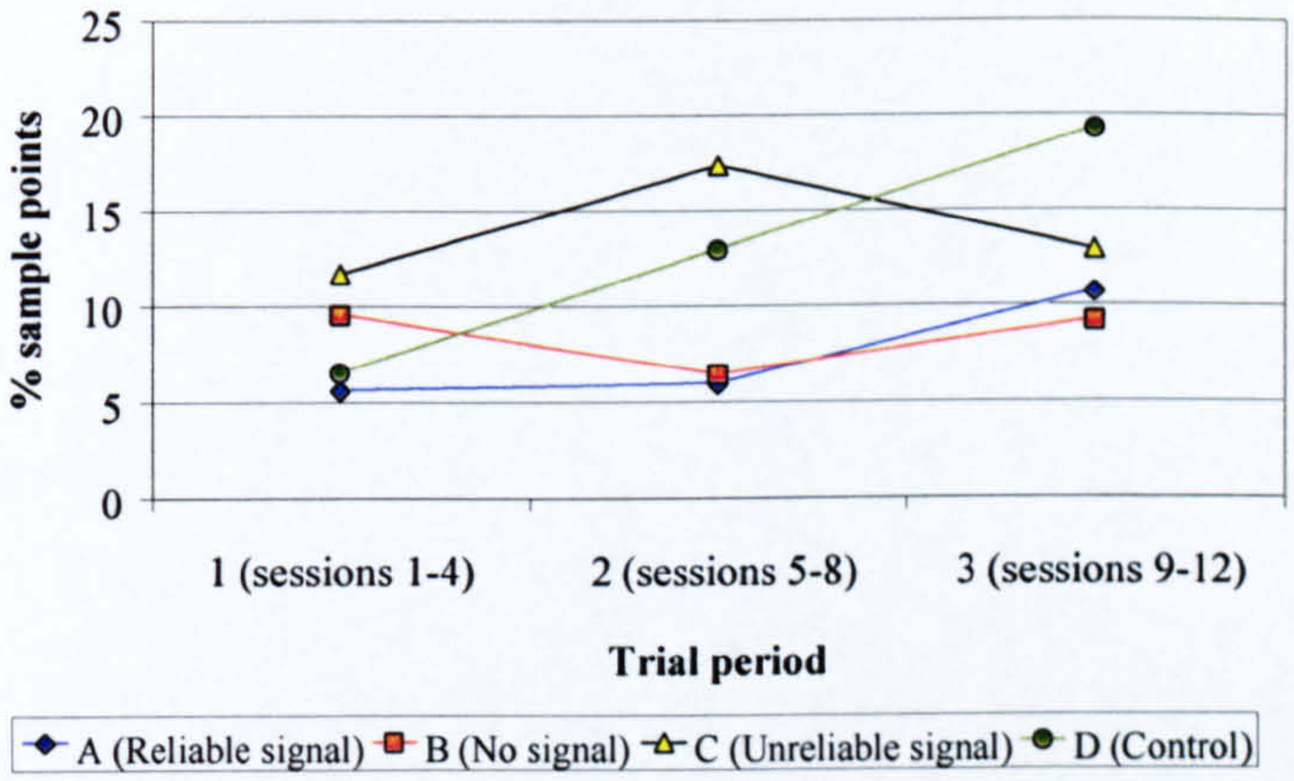
Interactions between condition, trial period and time period of session

Interactions between 'Trial Period' and 'Signalled Predictability'
There were significant interactions between 'Trial Period' and 'Signalled Predictability' for 'Inactive (not watching observer)' and 'Inactive (watching observer)' (see Table A1.7). Significant interactions are described.

Inactive (not watching observer)

There was a significant interaction between trial and condition for this behaviour (see Figure A1.7 and Table A1.7). Amount of inactivity while not watching the observer increased over trials in Condition B (the control condition). Amounts of this behaviour in Conditions A and B were similar throughout the three trial periods; in Condition C, the behaviour increased during trial period 2, but decreased again, to levels similar to those in Conditions B and C, during Trial Period 3.

Figure A1.7 - Interaction between trial and condition for Inactive (Not watching observer)



Inactive (watching observer)

There was significant interaction between trial and condition (Figure A1.8 and Table A1.7). The amount of inactivity while watching the observer increased over trials in Condition A, and decreased in Condition C. There was no apparent overall trend for Conditions B and D.

Figure A1.8 - Interaction between trial and condition for Inactive (watching observer)

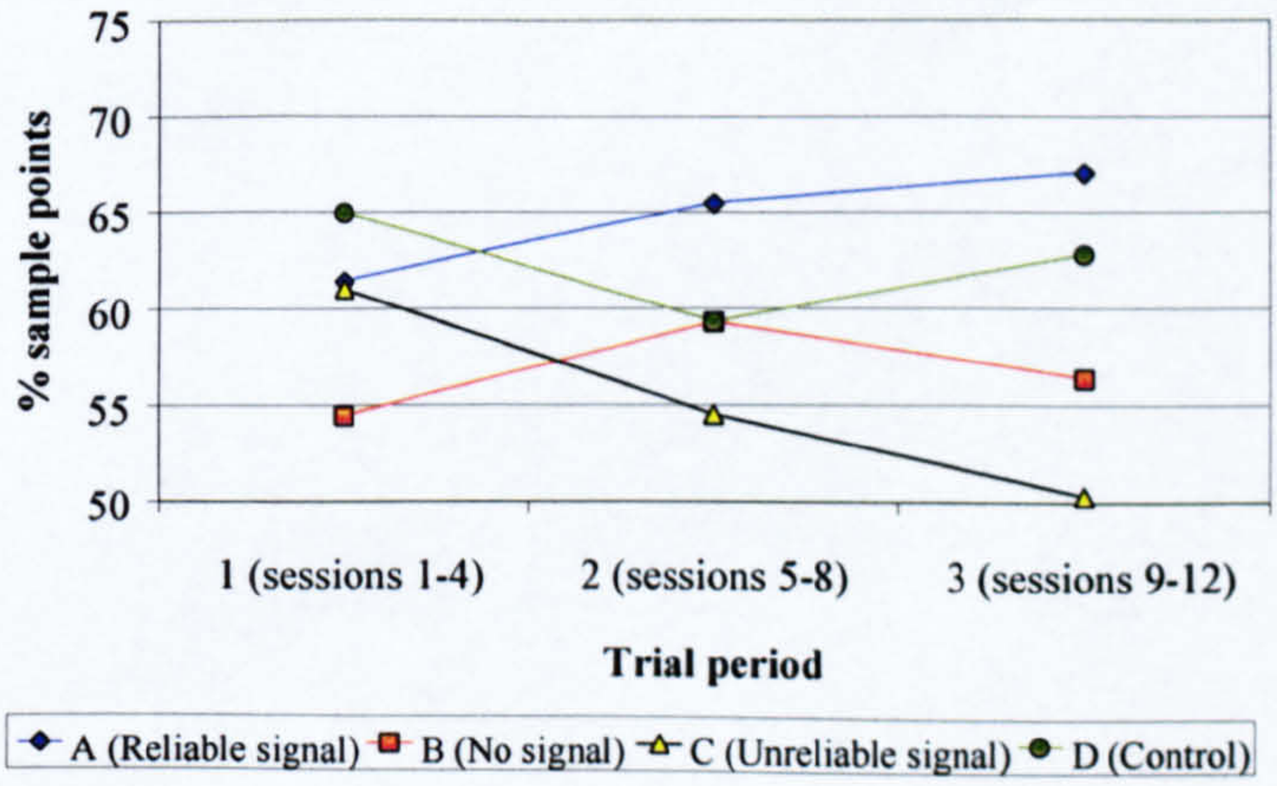


Table A1.7 Results of ANOVAs for interaction between 'Trial Period' and 'Signalled Predictability' on all behaviours

Behaviour	F	p
Inactive (not watching observer)	6.26	<0.001***
Inactive (watching observer)	3.98	<0.01**
Vocalise	0.83	0.56

All d.f. = 6,40

Interactions between 'Signal Period' and 'Signalled Predictability'

There was a significant interaction between 'Signal Period' and 'Signalled Predictability' for 'Inactive (watching observer)'. This is described.

Inactive (watching observer)

There was a significant interaction for signal and condition (Figure A1.9 and Table A1.8). For Conditions A and C, amounts of inactivity while watching the observer were greatest in the second signal period, *i.e.* in the period after the signal but before the food. There did not appear to be an effect of signal period on Conditions B and D.

Figure A1.9 - Interaction between signal and condition for Inactive (watching observer)

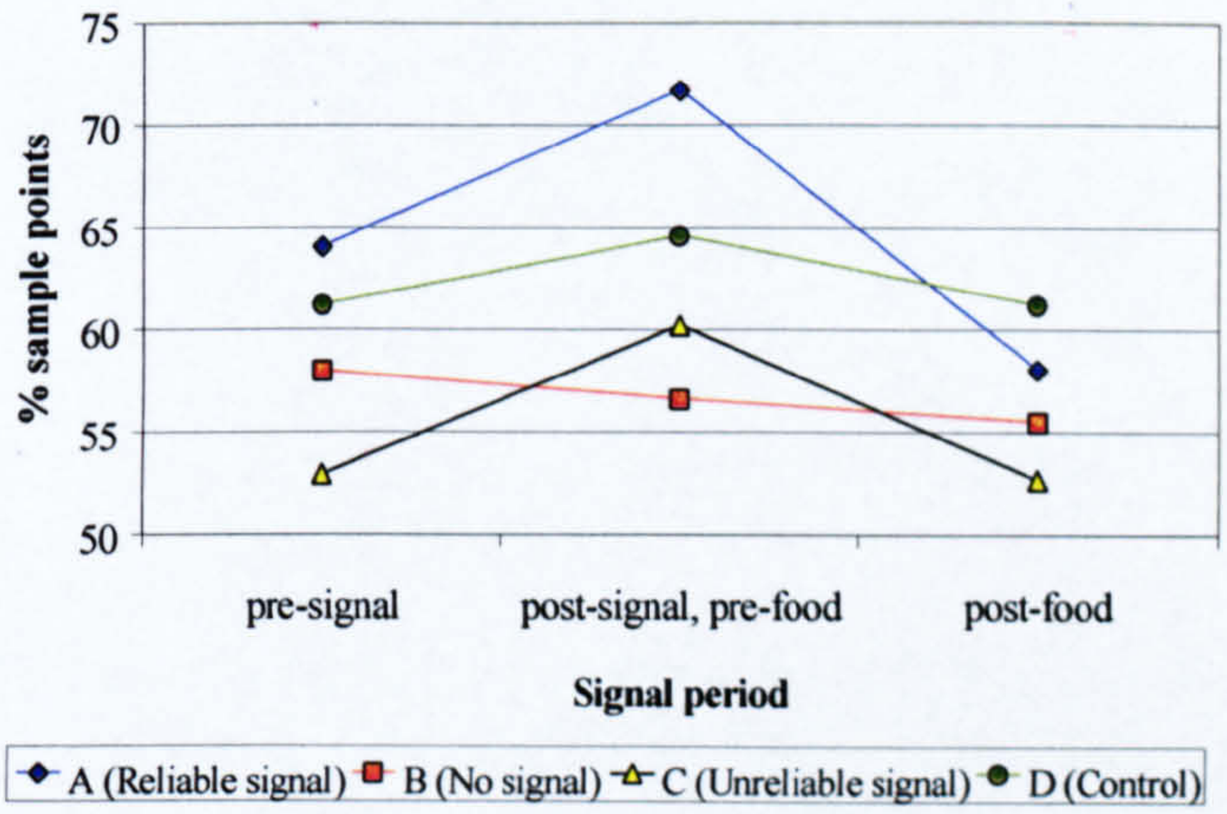


Table A1.8 Results of ANOVAs for interaction between 'Signal Period' and 'Signalled Predictability' on all behaviours

Behaviour	F	p
Inactive (not watching observer)	1.30	0.28
Inactive (watching observer)	2.36	<0.05*
Vocalise	0.46	0.83

All d.f. = 6,40

Interactions between 'Trial Period' and 'Signal Period'
There were no significant interactions between 'Trial Period' and 'Signal Period' (see Table A1.9).

Table A1.9 Results of ANOVAs for interaction between 'Trial Period' and 'Signal Period' on all behaviours

Behaviour	F	p
Inactive (not watching observer)	2.11	0.09
Inactive (watching observer)	2.46	0.06
Vocalise	0.51	0.73

All d.f. = 4,12

Three-way interactions between 'Signalled Predictability', 'Trial Period' and 'Signal Period'
There were no significant three-way interactions between 'Signalled Predictability', 'Trial Period' and 'Signal Period' (see Table A1.10).

Table A1.10 Results of ANOVAs for three-way interaction between 'Trial Period', 'Signal Period' and 'Signalled Predictability' on all behaviours

Behaviour	F	p
Inactive (not watching observer)	1.36	0.20
Inactive (watching observer)	1.03	0.43
Vocalise	1.12	0.35

All d.f. = 12,80

DISCUSSION

Inactive (not watching the observer)

Animals in condition A (reliable signal) spent the least time inactive but not watching the observer. The amount of time spent inactive but not watching the observer increased over trials for the control condition, but there was no overall increase or decrease for any of the other conditions. This is possibly due to habituation to the observer, who never interacted with the animals in the control condition in any way, unlike the other conditions, in which the animals were all given food at varying frequencies.

Inactive (watching the observer)

The amount of time spent inactive and watching the observer for animals in conditions A and C followed the same pattern as for general inactivity. There was also an effect of signal, again similar to that seen in the general activity category, with levels being higher in the post-signal, pre-food period for both the conditions, A and C, that received a signal. This supports the idea that increased inactivity was due to increased vigilance associated with an expected food delivery. There was no overall effect of trial period on this behaviour. However, the amount of time spent

watching the observer increased over trials for condition A (reliable signal). This is difficult to explain, as the 'safety-signal' hypothesis might suggest that because the food was predictable, vigilance could be reduced for the 'safe period' of the trial (i.e. the period before, and up to one minute after the signal), when the food would not be delivered. Conversely, the amount of time spent inactive and watching the observer increased over trials for condition C (unreliable signal). However, the time spent watching the observer was only recorded if the animal was inactive, so it is perhaps not a reliable indicator of vigilance. The animals may equally have been watching the observer whilst being active, *e.g.* whilst scratching or vocalising. A possible reason for the unexpected effects seen in conditions A (reliable signal) and C (unreliable signal) may be the same as discussed for the 'inactive (all categories)' behaviour. Increasing arousal, associated with the unpredictable food, may be responsible for the decrease in inactivity seen over trials for condition C (unreliable signal).

The increase in inactivity whilst watching the observer may be analogous to the increased inactivity seen in chimpanzees fed on a predictable schedule, which seemed to be 'waiting' for their meal to arrive (Bloomsmith & Lambert, 1995). When these chimpanzees were fed on an unpredictable schedule, an increase in species-specific activities was seen, which was thought to improve their welfare. The decrease in inactivity seen in this study when an unreliable signal precedes feeding might also indicate enhanced welfare. However, before concluding that feeding primates on an unpredictable schedule, or in the presence of unreliable signals, is beneficial to their welfare, it is important to find out the reason for the decrease in inactivity seen. If the decreased inactivity is due to increased arousal, it may actually be detrimental.

3. Vocalising

In the present study, the only significant result was the effect of trial; levels of vocalisation decreased as trials progressed. This may be due to habituation to the experimental procedure occurring over trials. There was no effect on this or any other behaviour of habituation during trials (as seen in the initial study presented in Chapter 5); however, habituation may have taken place over the whole trial period.

Inactive (not watching observer), Inactive (watching observer) and Vocalise

There was no significant main effect of predictability of food delivery on these categories of behaviour (see Table A2.1 and Figures A2.1 and A2.2).

Table A2.1 Results of ANOVAs for effects of predictability of food on all behaviours

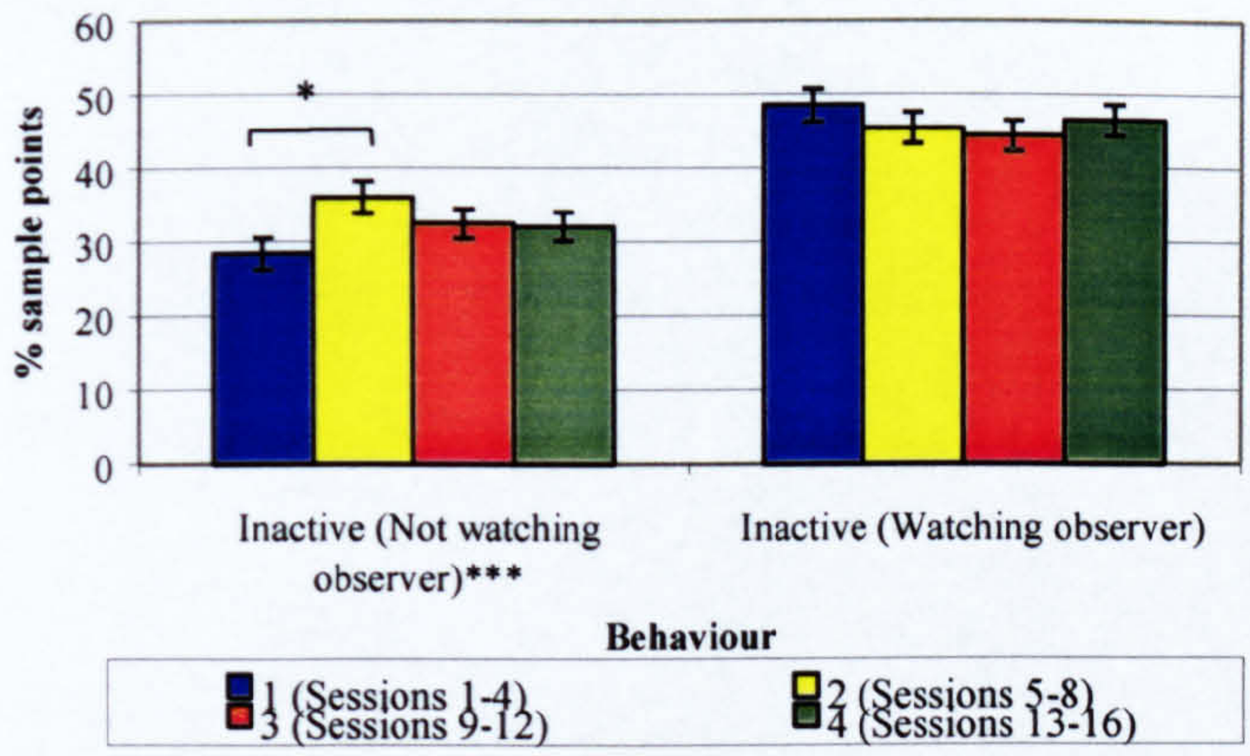
Behaviour	**F**	**p**
Inactive (not watching observer)	2.28	0.09
Inactive (watching observer)	0.33	0.86
Vocalising	2.68	0.06

All d.f.=4,25

Effects of Trial Period on behaviour

Each behavioural category is again presented separately. Where a significant main effect was found, results of post-hoc paired *t*-tests (with the Bonferroni correction) are given.

Figure A2.3 Percentage sample points spent inactive in the four trial periods (bars represent Standard Errors)



*p<0.05; **p<0.01; ***p<0.001

Asterisks by x-axis labels indicate significance of ANOVAs. Asterisks above boxes indicate significance of post-hoc pairwise *t*-tests (following Bonferroni correction).

Inactive (not watching observer)

There was a significant main effect of trial period on this behaviour (see Table A2.2). The only significant difference between the individual trial periods was that there was significantly more inactivity (not watching observer) in Trial Period 2 than Trial Period 1 (see Table A2.3 and Figure A2.3).

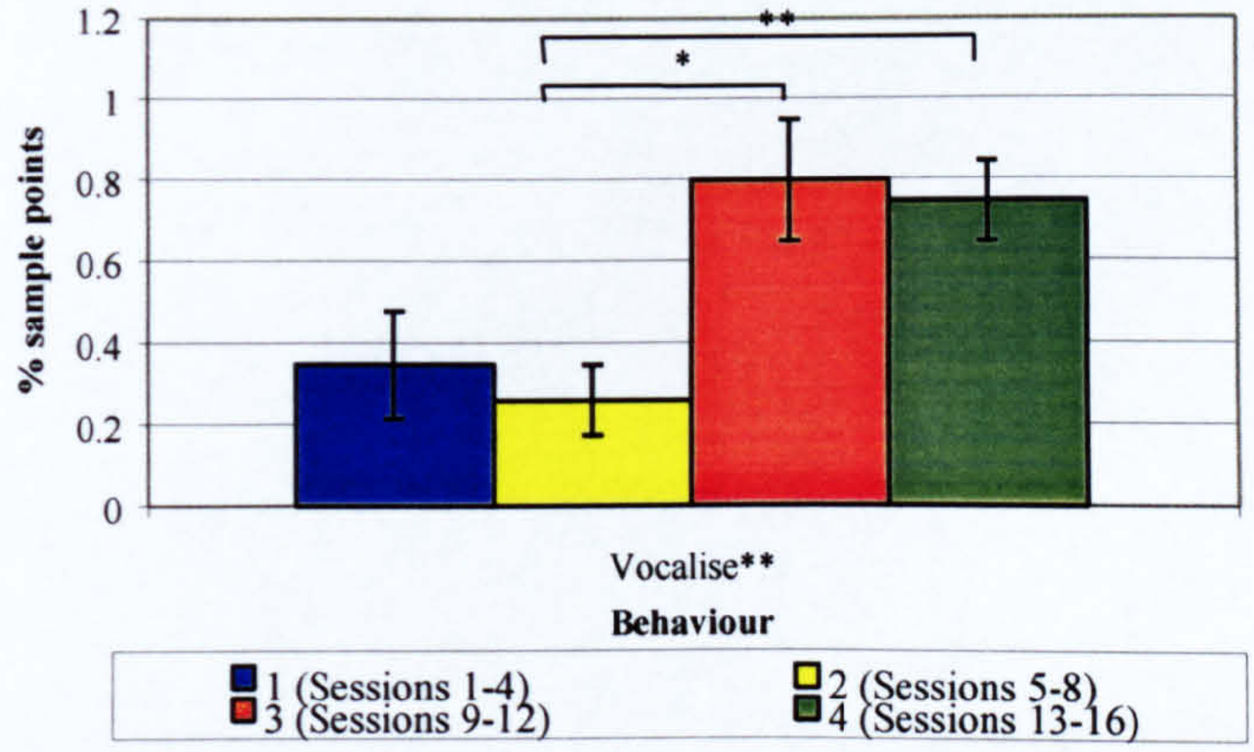
Inactive (watching observer)

There was no significant main effect of predictability of food delivery on this behaviour (see Table A2.2 and Figure A2.3).

Vocalise

There was a significant main effect of Trial Period on this behaviour (see Table A2.2). There was significantly more scent marking in Trial Periods 3 and 4 than Trial Period 2 (see Table A2.3 and Figure A2.4).

Figure A2.4 Percentage sample points spent scratching, scent marking and foraging in the four trial periods



*p<0.05; **p<0.01

Asterisks by x-axis labels indicate significance of ANOVAs. Asterisks above boxes indicate significance of post-hoc pairwise *t*-tests (following Bonferroni correction).

Table A2.2 Results of ANOVAs for effects of trial period on all behaviours

Behaviour	F	p
Inactive (not watching observer)	6.98	<0.001***
Inactive (watching observer)	1.87	0.14
Vocalising	5.52	<0.01**

All d.f.=2,50

Table A2.3 Post-hoc *t*-test *t* and p values for mean percentage sample points spent in each behaviour in each trial period (only behaviours showing a significant main effect of 'trial period' included)

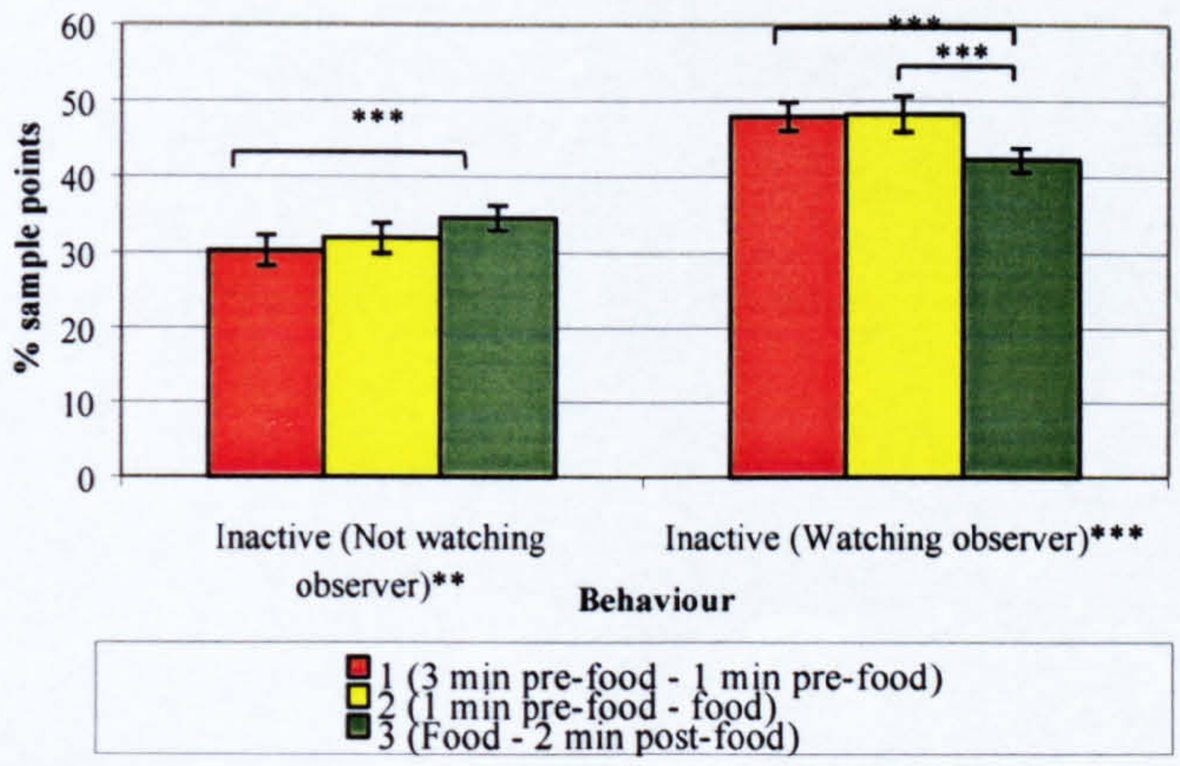
Behaviour	Trial period	*t*	p (uncorrected)	p (following Bonferroni correction)
Inactive (not watching observer)	1-2	4.11	<0.001***	<0.001***
	1-3	1.85	0.08	0.45
	1-4	1.77	0.09	0.53
	2-3	2.51	<0.05*	0.11
	2-4	2.65	<0.05*	0.08
	3-4	0.24	0.81	1.00
Vocalising	1-2	0.52	0.61	1.00
	1-3	2.28	<0.05*	0.18
	1-4	2.18	<0.05*	0.22
	2-3	3.43	<0.01**	<0.05*
	2-4	3.56	<0.001***	<0.01**
	3-4	0.29	0.77	1.00

All d.f. = 29

Effects of Time Period in relation to food delivery on behaviour

Each behavioural category is again presented separately. Where a significant main effect was found, results of post-hoc pairwise *t*-tests (with the Bonferroni correction) are given.

Figure A2.5 Percentage sample points spent inactive in the three time periods relative to food delivery (bars represent Standard Errors)



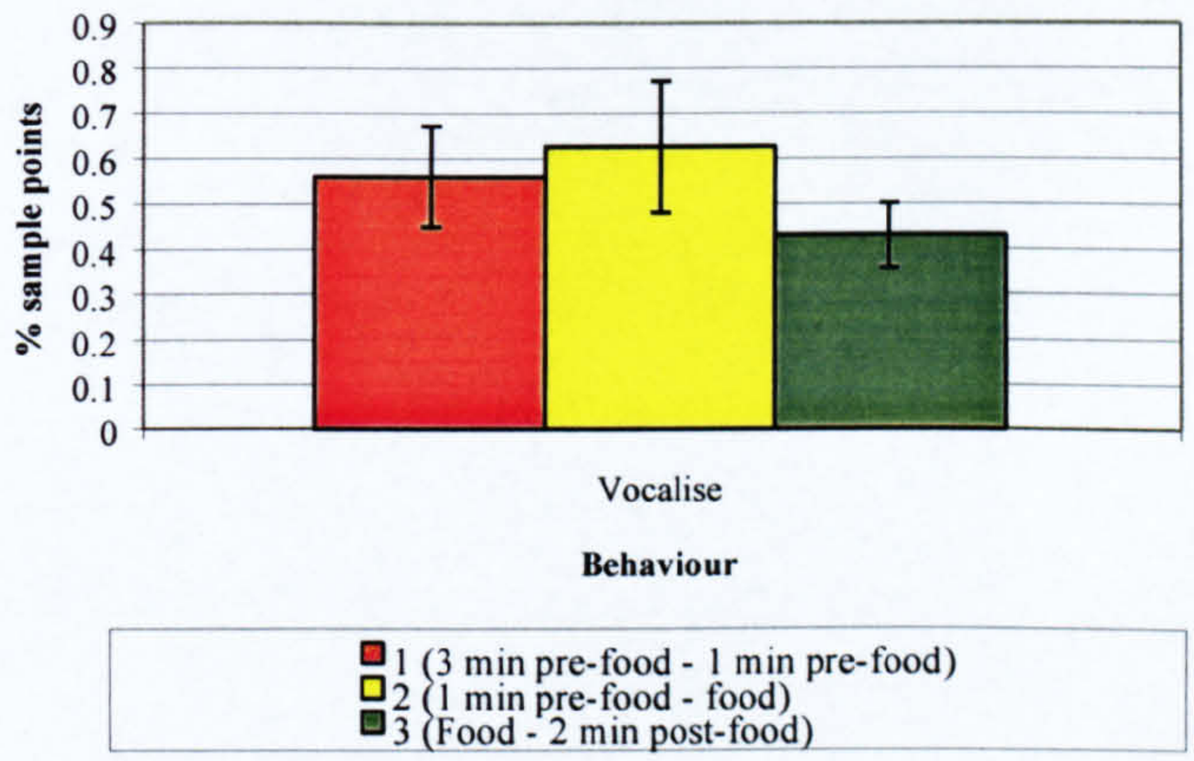
*p<0.05; **p<0.01; ***p<0.001

Asterisks by x-axis labels indicate significance of ANOVAs. Asterisks above boxes indicate significance of post-hoc pairwise *t*-tests (following Bonferroni correction).

Inactive (not watching observer)
There was a significant main effect of Time Period on this behaviour (see Table A2.4). There was significantly more inactivity (not watching the observer) in Time Period 3 than Time Period 1 (see Table A2.5 and Figure A2.5).

Inactive (watching observer)
There was a significant main effect of Time Period on this behaviour (see Table A2.4). There was significantly more inactivity (watching the observer) in Time Periods 1 and 2 than Time Period 3 (see Table A2.5 and Figure A2.5).

Figure A2.6 Percentage sample points spent vocalising in the three time periods relative to food delivery (bars represent Standard Errors)



Vocalise
There was no significant main effect of Time Period on this behaviour (see Figure A2.6 and Table A2.4).

Table A2.4 Results of ANOVAs for effects of time in relation to food delivery on all behaviours

Behaviour	**F**	**p**
Inactive (not watching observer)	8.86	<0.01**
Inactive (watching observer)	15.14	<0.001***
Vocalise	0.76	0.47

All d.f.=3,75

Table A2.5 Post-hoc *t*-test *t* and p values for mean percentage sample points spent in each behaviour in each time period in relation to food delivery (only behaviours showing a significant main effect of 'time period' included)

Behaviour	Trial period	*t*	p (uncorrected)	p (following Bonferroni correction)
Inactive (not watching observer)	1-2	1.92	0.06	0.19
	1-3	3.97	<0.001***	<0.001***
	2-3	2.06	<0.05*	0.14
Inactive (watching observer)	1-2	0.27	0.79	1.00
	1-3	4.72	<0.001***	<0.001***
	2-3	3.63	<0.001***	<0.01**

All d.f. = 29

Effects of interactions between the three variables
Interactions between 'Trial Period' and 'Predictability of food delivery'

There were significant interactions between 'Trial Period' and 'Predictability of food delivery' on 'Inactive (not watching observer)', 'Inactive (watching observer)' and 'Vocalise' (see Table A2.6).

Table A2.6 Results of ANOVAs for interaction between 'Trial Period' and 'Predictability of food delivery' on all behaviours (all trials)

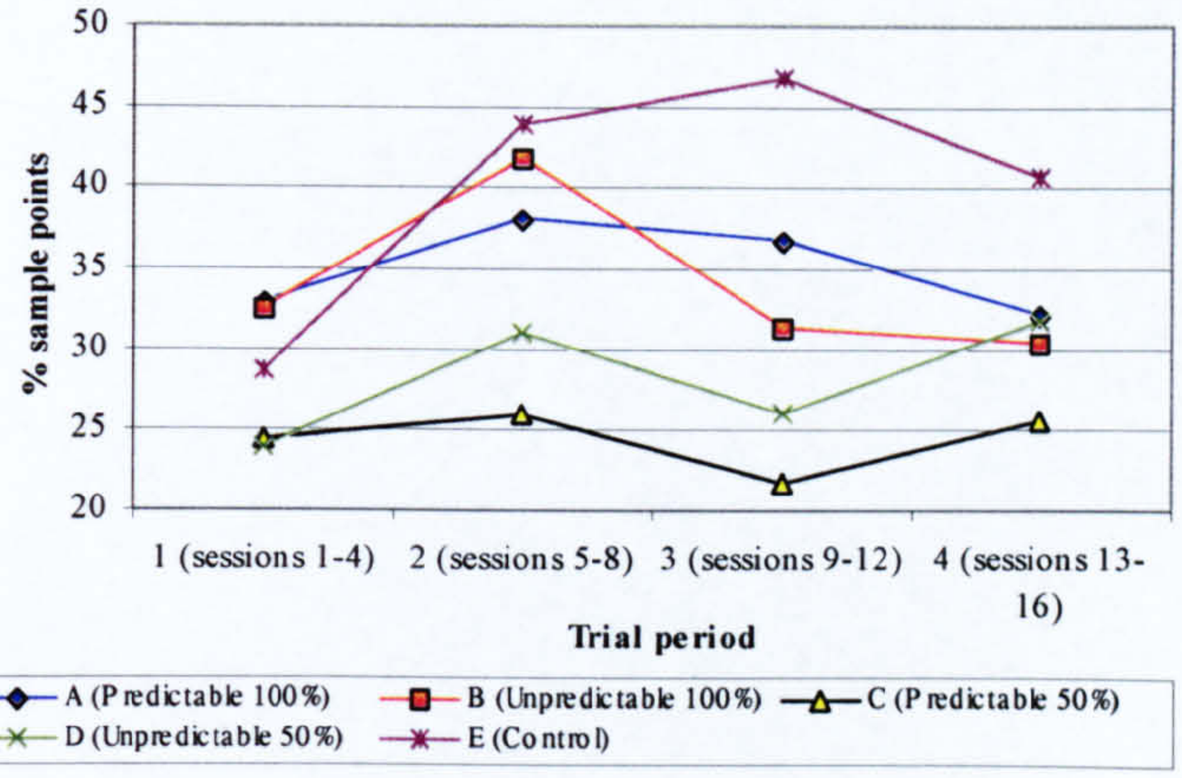
Behaviour	F	p
Inactive (not watching observer)	2.44	<0.05*
Inactive (watching observer)	2.71	<0.01**
Vocalising	6.73	<0.001***

All d.f.=12,75

Inactive (not watching observer)

There was an increase in inactivity (not watching observer) between Trial Periods 1 and 2 for Condition B and the Control condition (Condition E). There was a decrease between trial periods 2 and 3 for Condition B, and rates remained fairly low for trial period 4. Amounts of this behaviour were greater in the Control condition than in any of the four experimental conditions for Trial Periods 3 and 4. Rates were lowest in Conditions C and D, and stayed relatively constant over trial periods for these conditions. Condition A animals showed an intermediate level of the behaviour (see Figure A2.7).

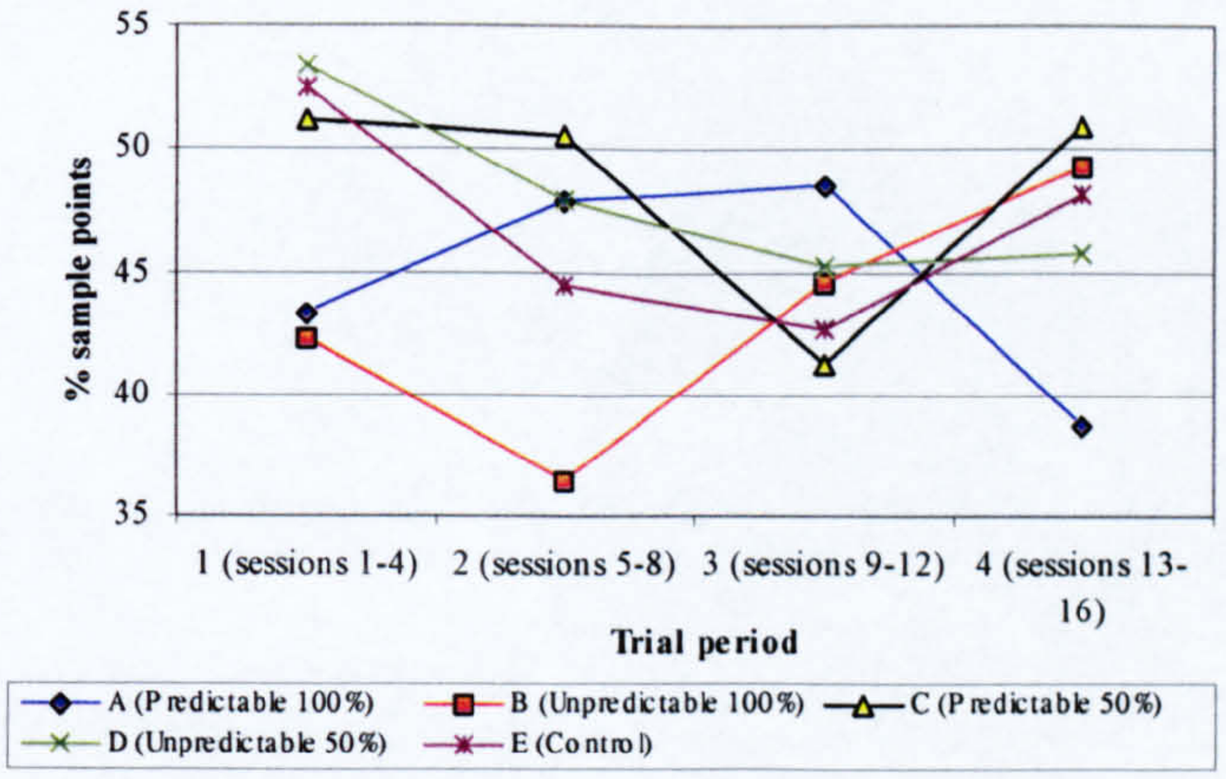
Figure A2.7 Interaction between 'Trial Period' and 'Predictability of food delivery' for 'Inactive (not watching observer)'



Inactive (watching observer)

There was a decrease in the rate of this behaviour over Trial Periods 1-3 with an increase at Trial Period 4 for the Control condition. This pattern was reversed for Condition A, with an increase over the first three trial periods and a decrease at Trial Period 4. At Trial Period 1, rates of inactivity (watching observer) were similar, and lowest, for Conditions A and B, while rates for Conditions C, D and E were similar. At Trial Period 4, animals in Condition A showed the lowest rates of the behaviour (see Figure A2.8).

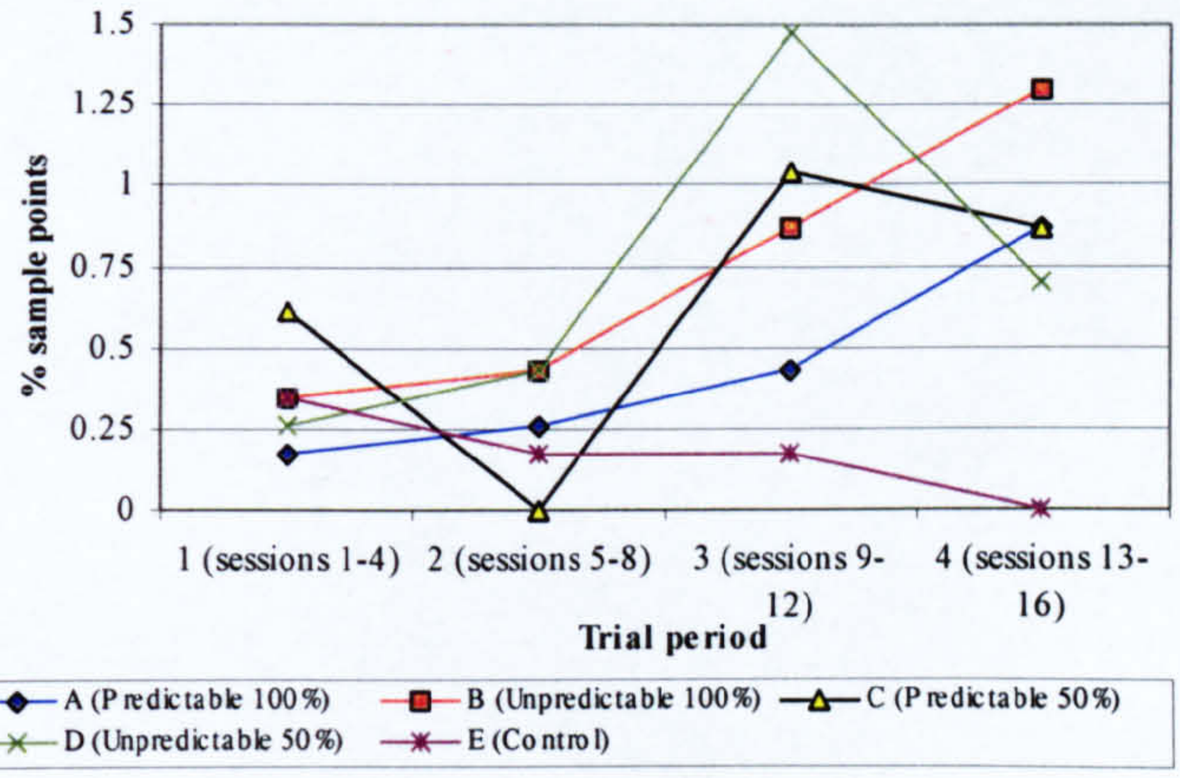
Figure A2.8 Interaction between ''Trial Period' and 'Predictability of food delivery' for 'Inactive (watching observer)'



Vocalise

Vocalising occurred at a fairly low rate for all five conditions at Trial Periods 1 and 2. At Trial Period 3, rates for Conditions A and E were still relatively low, but rates for Conditions B, C and D had increased. At Trial Period 4, rates in the Control condition were zero, at an intermediate level for Conditions A, C and D, and at a high rate for Condition B (see Figure A2.9).

Figure A2.9 Interaction between 'Trial Period' and 'Predictability of food delivery' for 'Vocalise'



Interactions between 'Time Period' and 'Predictability of food delivery'

There were significant interactions between 'Time Period' and 'Predictability of food delivery' on 'Inactive (watching observer)' and 'Vocalise' (see Table A2.7).

Table A2.7 Results of ANOVAs for interaction between 'Time Period' and 'Predictability of food delivery' on all behaviours

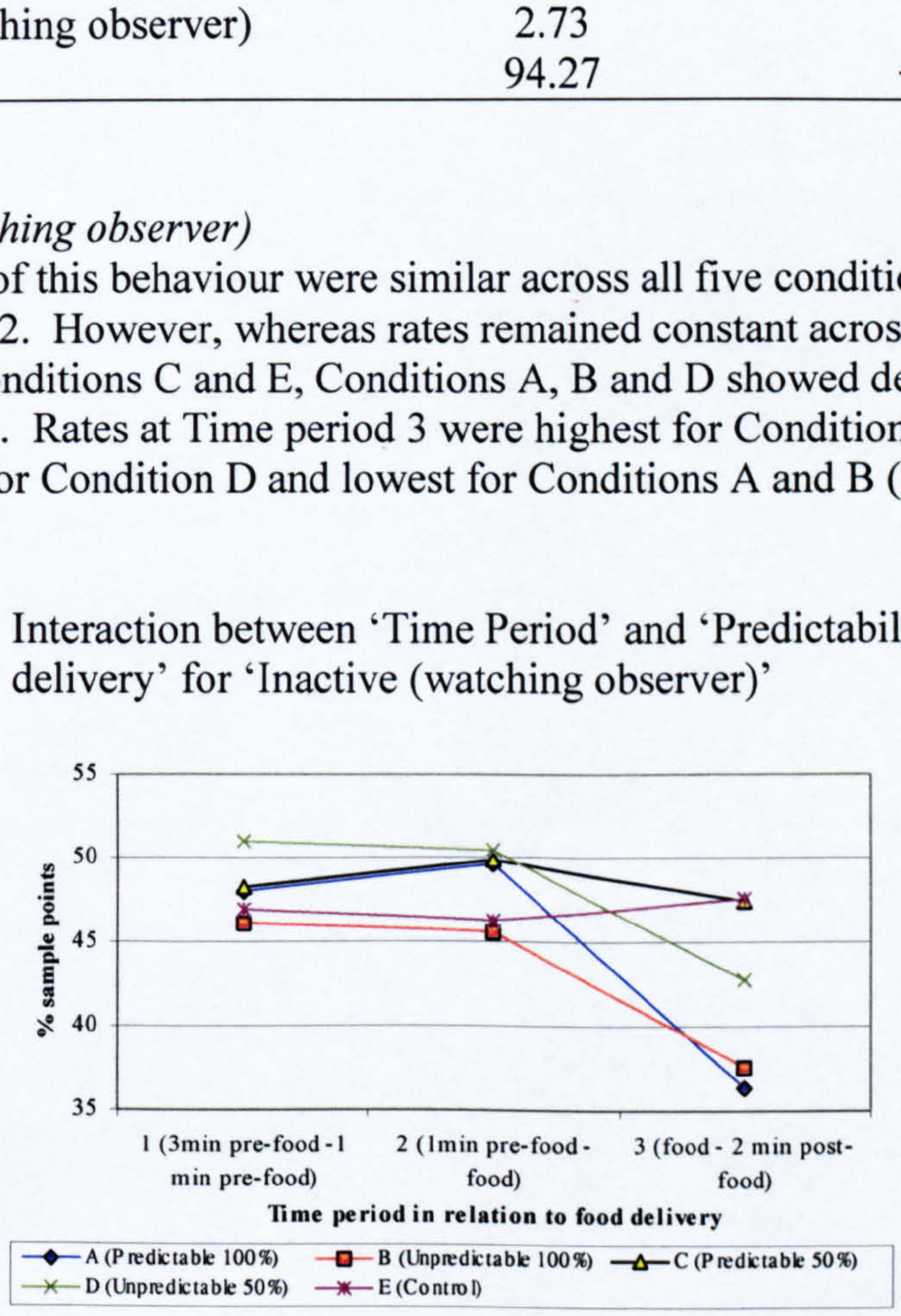
Behaviour	F	p
Inactive (not watching observer)	1.84	0.09
Inactive (watching observer)	2.73	<0.05*
Vocalising	94.27	<0.001***

All d.f.= 8,50

Inactive (watching observer)

Rates of this behaviour were similar across all five conditions for Time Periods 1 and 2. However, whereas rates remained constant across all three time periods for Conditions C and E, Conditions A, B and D showed decreases in rates at Time Period 3. Rates at Time period 3 were highest for Conditions C and E, intermediate for Condition D and lowest for Conditions A and B (see Figure A2.10).

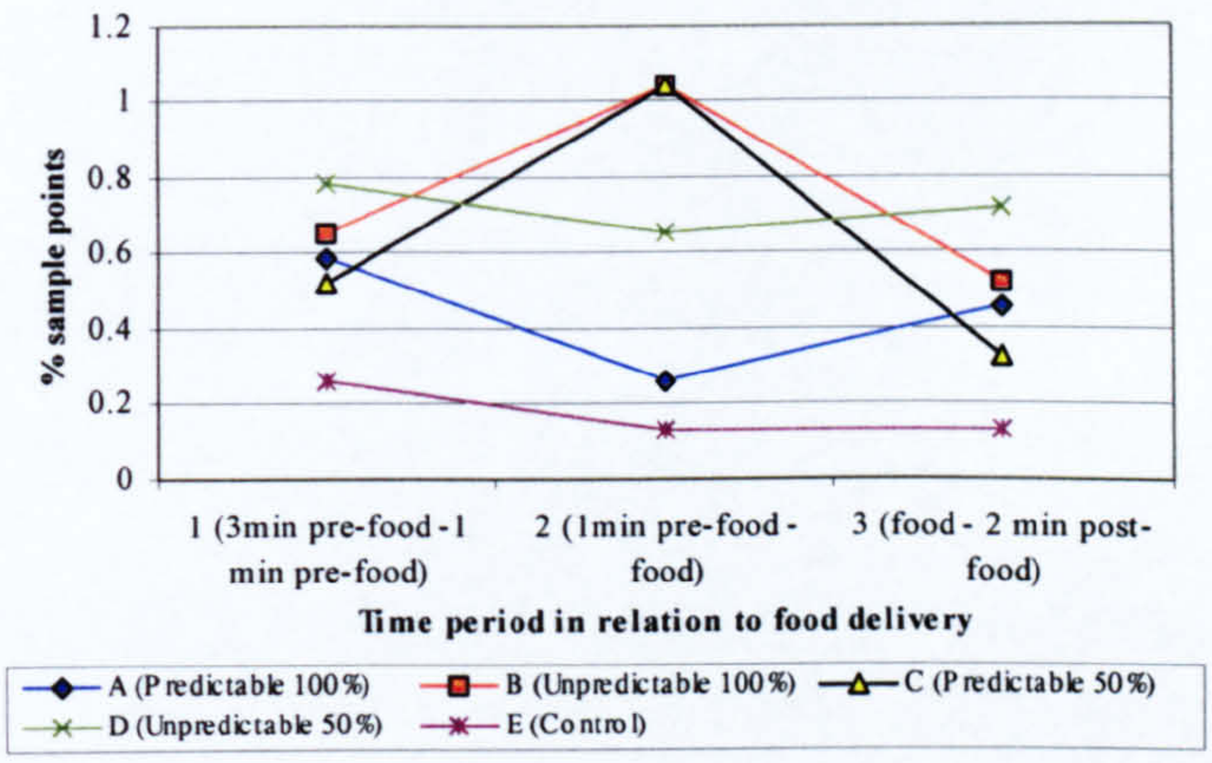
Figure A2.10 Interaction between 'Time Period' and 'Predictability of food delivery' for 'Inactive (watching observer)'



Vocalise

Rates of this behaviour were relatively constant across all three time periods for Conditions D and E, with animals in Condition E showing more vocalising than those in Condition E in all time periods. Rates for Condition A animals were similar in Time Periods 1 and 3, with reduced rates in Time Period 2. The opposite pattern was seen for animals in Conditions B and C, which again showed similar rates in Time Periods 1 and 3, but with increased rates in Time Period 2 (see Figure A2.11).

Figure A2.11 Interaction between 'Time Period' and 'Predictability of food delivery' for 'Vocalise'



Interactions between 'Trial Period' and 'Time Period'

There were significant interactions between 'Trial Period' and 'Time Period' for 'Inactive (not watching observer)', 'Inactive (watching observer)' and 'Vocalise' (see Table A2.8).

Table A2.8 Results of ANOVAs for interaction between 'Time Period' and 'Trial Period' on all behaviours (all trials)

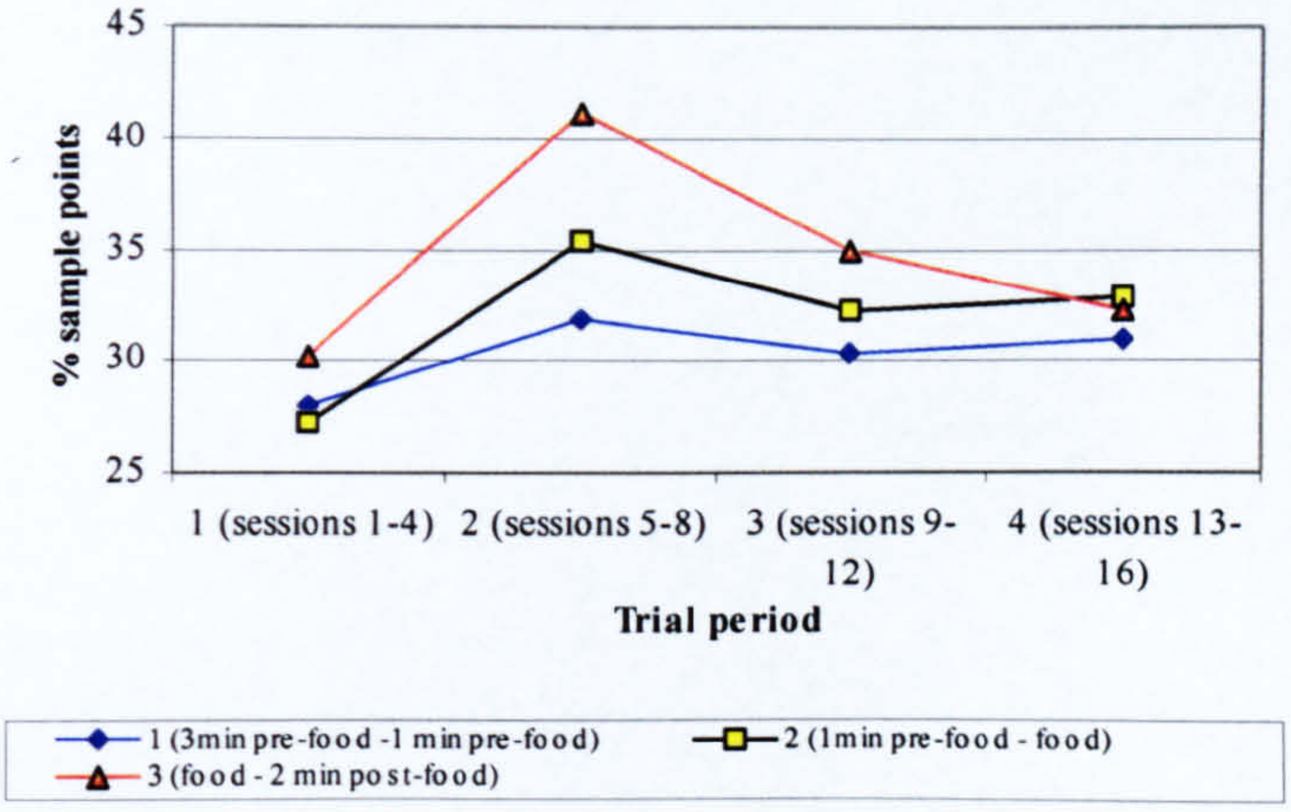
Behaviour	F	p
Inactive (not watching observer)	2.41	<0.05*
Inactive (watching observer)	2.71	<0.05*
Vocalising	78.96	<0.001***

All d.f.= 6,150

Inactive (not watching observer)

The lowest rates of inactivity (not watching observer) occurred in Trial Period 1, where there was little difference between the rates for the three separate time periods. There was an increase in rates of this behaviour in all three time periods for Trial Period 2. The greatest increase occurred for Time Period 3, the least for Time Period 1, with Time Period 2 showing an intermediate increase. Rates of the behaviour decreased in all three time periods at Trial Period 3, where the differences between the three time periods had lessened. There were no apparent differences in rates of the behaviour between the three time periods at Trial Period 4 (see Figure A2.12).

Figure A2.12 Interaction between 'Time Period' and 'Trial Period' for 'Inactive (not watching observer)'

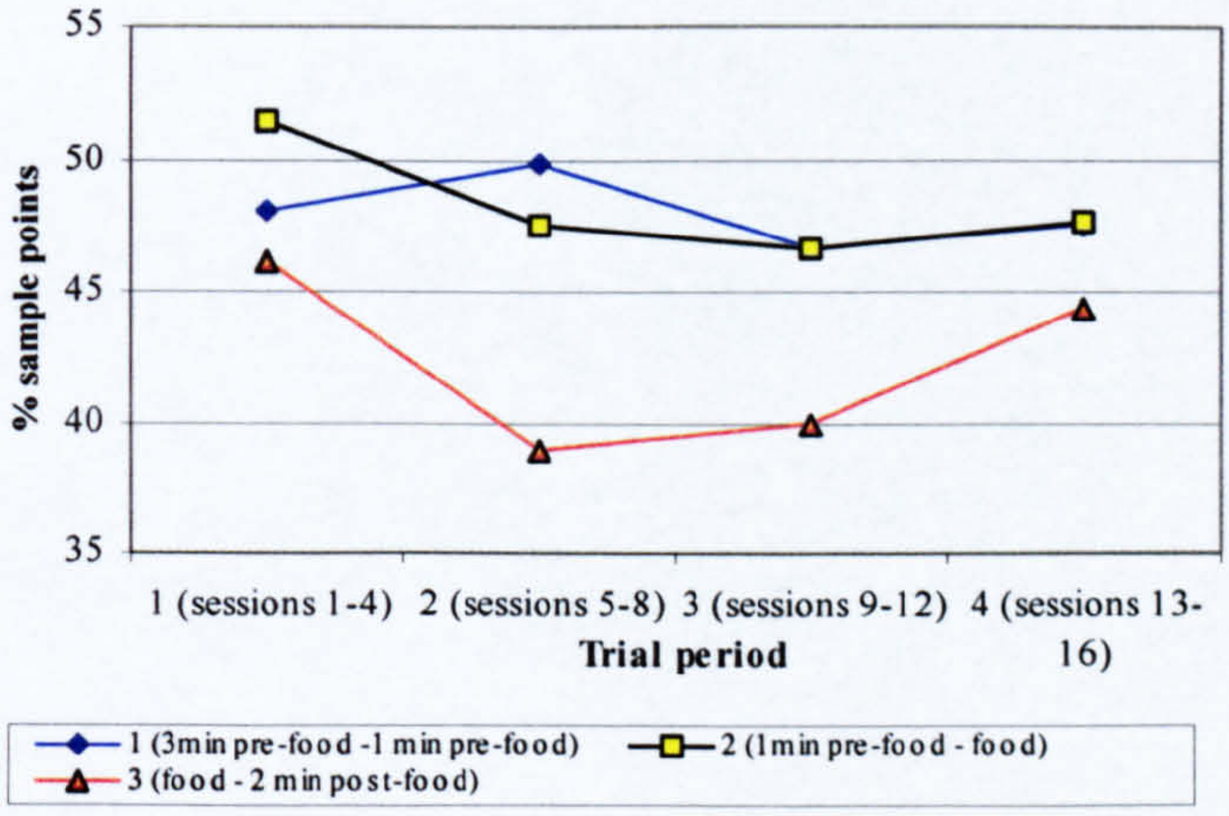


Inactive (watching observer)

Rates of inactivity (watching observer) remained relatively constant across the four trial periods for Time Period 1. Rates were slightly higher in Trial Period 1 than the other three trial periods for Time Period 2, and similarly constant over Trial Periods 2, 3 and 4.

However, Time Period 3 showed the inverse pattern to that seen for 'Inactive (not watching observer)'. The lowest rates of inactivity (watching observer) were seen in Trial Period 1, and the greatest in Trial Period 2. An intermediate rate was seen by Trial Period 3, and the rate had decreased (to a level only slightly higher than Trial Period 1) by Trial Period 4 (see Figure A2.13).

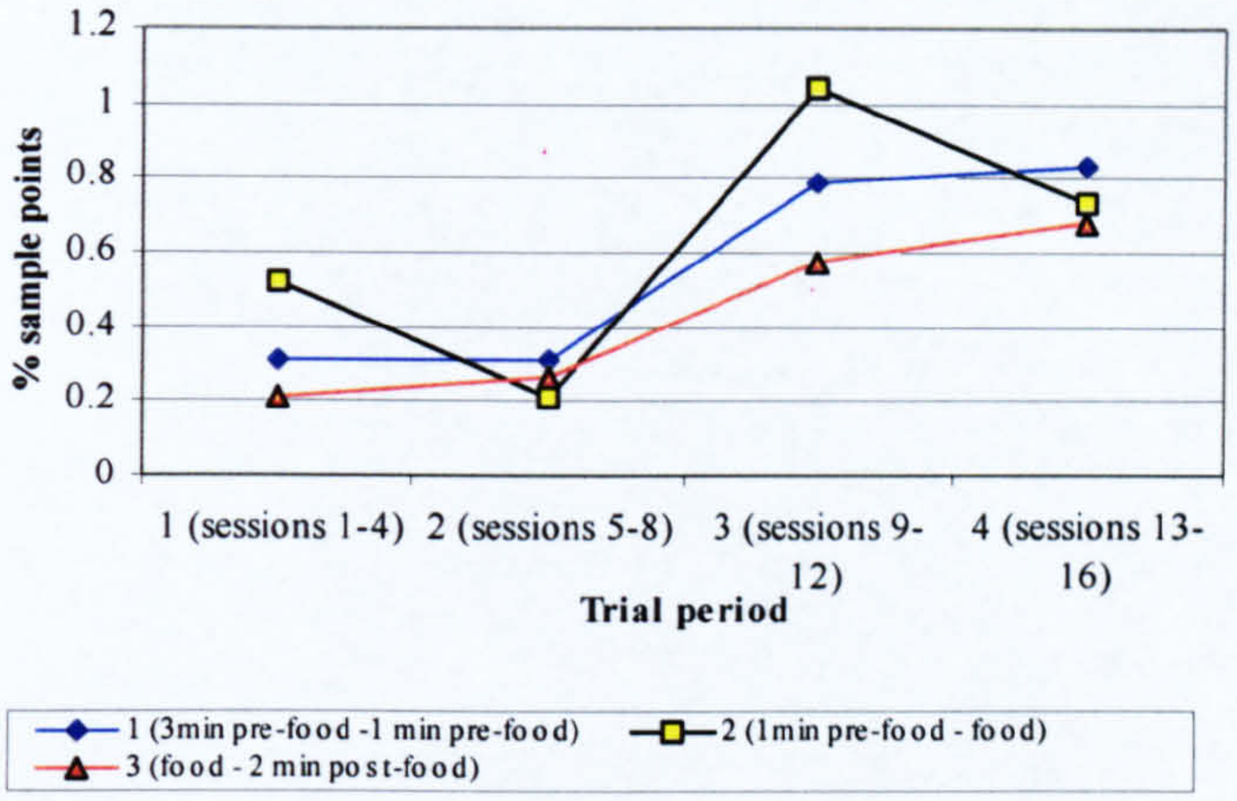
Figure A2.13 Interaction between 'Time Period' and 'Trial Period' for 'Inactive (watching observer)'



Vocalise

Similar patterns for vocalising were seen in Time Periods 1 and 3. For both these time periods, rates remained constant, and lowest, between Trial Periods 1 and 2. An increase in rates of vocalising was seen at Trial Period 3, and rates remained similar to this at Trial Period 4. However, for Time Period 2, rates at Trial Period 2 were decreased from those at Trial Period 1. Rates increased to a peak at Trial Period 3, and decreased again at Trial period 4 (see Figure A2.14).

Figure A2.14 Interaction between 'Time Period' and 'Trial Period' for 'Vocalise'



Three-way interactions between 'Predictability of food delivery','Trial Period' and 'Time Period'

There was a three-way interaction between 'Predictability of food delivery','Trial Period' and 'Time Period' on 'Vocalise' (see Table A2.9).

Table A2.9 Results of ANOVAs for interaction between signal, 'Trial Period' and 'Predictability of food delivery' on all behaviours (all trials)

Behaviour	F	p
Inactive (not watching observer)	0.53	0.96
Inactive (watching observer)	0.74	0.81
Vocalising	9.06	<0.001***

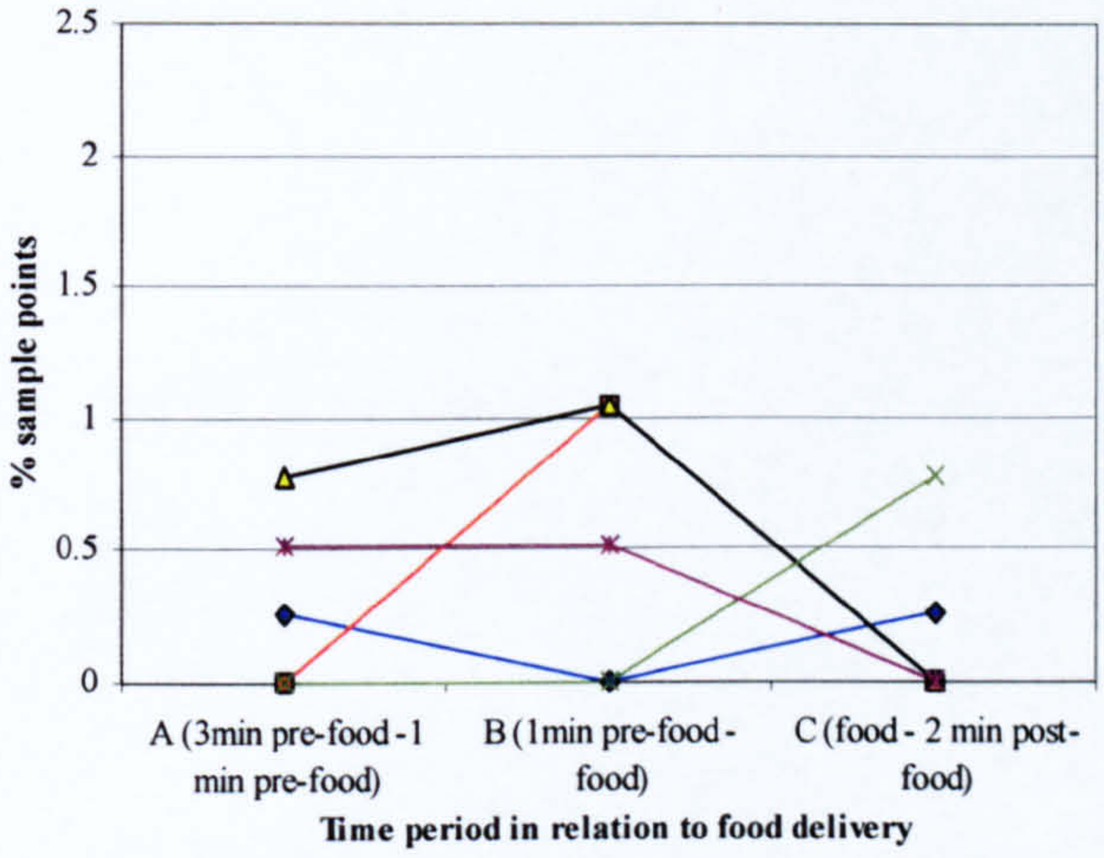
All d.f.= 24,150

Vocalise

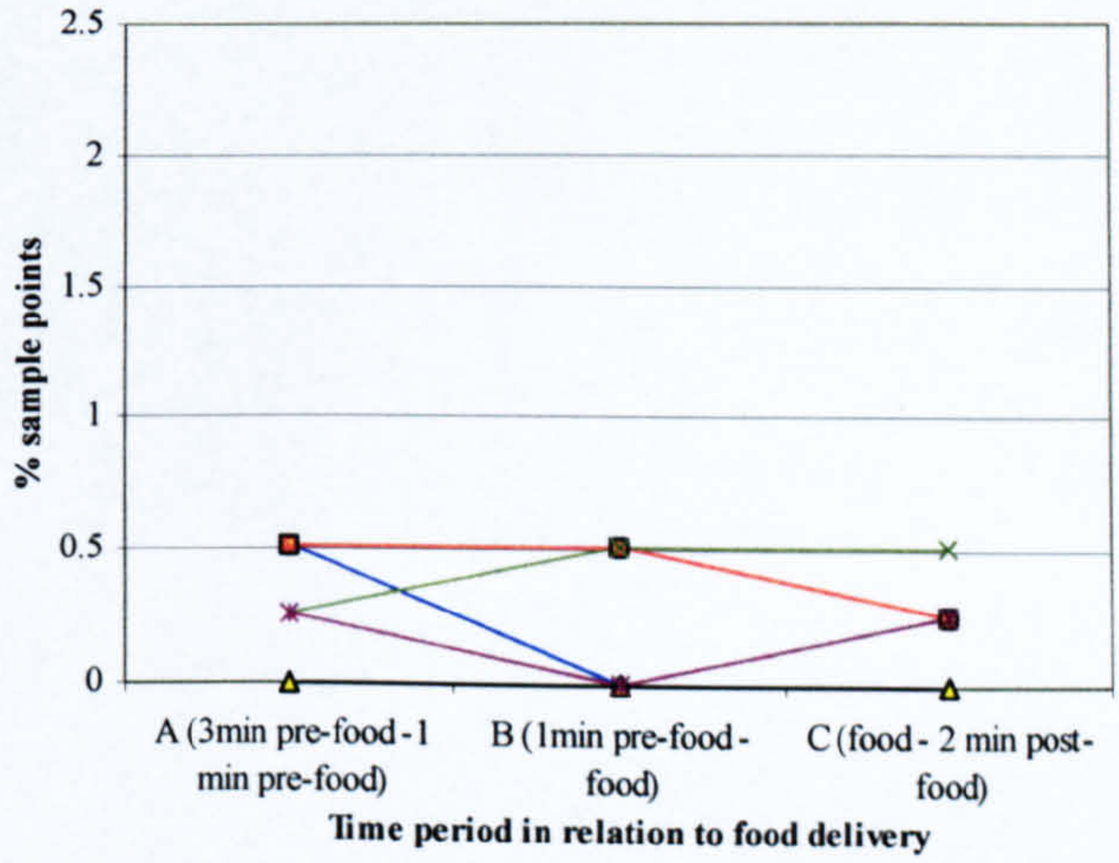
Rates of vocalising were fairly low, and similar across all time and trial periods for the Control condition. Rates for Condition A animals were similar to those seen in the Control condition for Trial Periods 1-3, but higher than Control rates for Trial Period 4. Condition B animals showed more vocalising in Trial Periods 3 and 4 than Trial Periods 1 and 2. Condition B and C animals tended to show greater rates of vocalising in Time Period 2 than Time Periods 1 and 3 for all Trial Periods. Vocalising peaked for Condition C animals at Time Period 2 in Trial Period 3, and for Condition D animals at Time Period 1 in Trial Period 3 (see Figure A2.15).

Figure A2.15 Interaction between 'Time Period' and 'Predictability of food delivery' for each of the four trial periods for 'Vocalise'

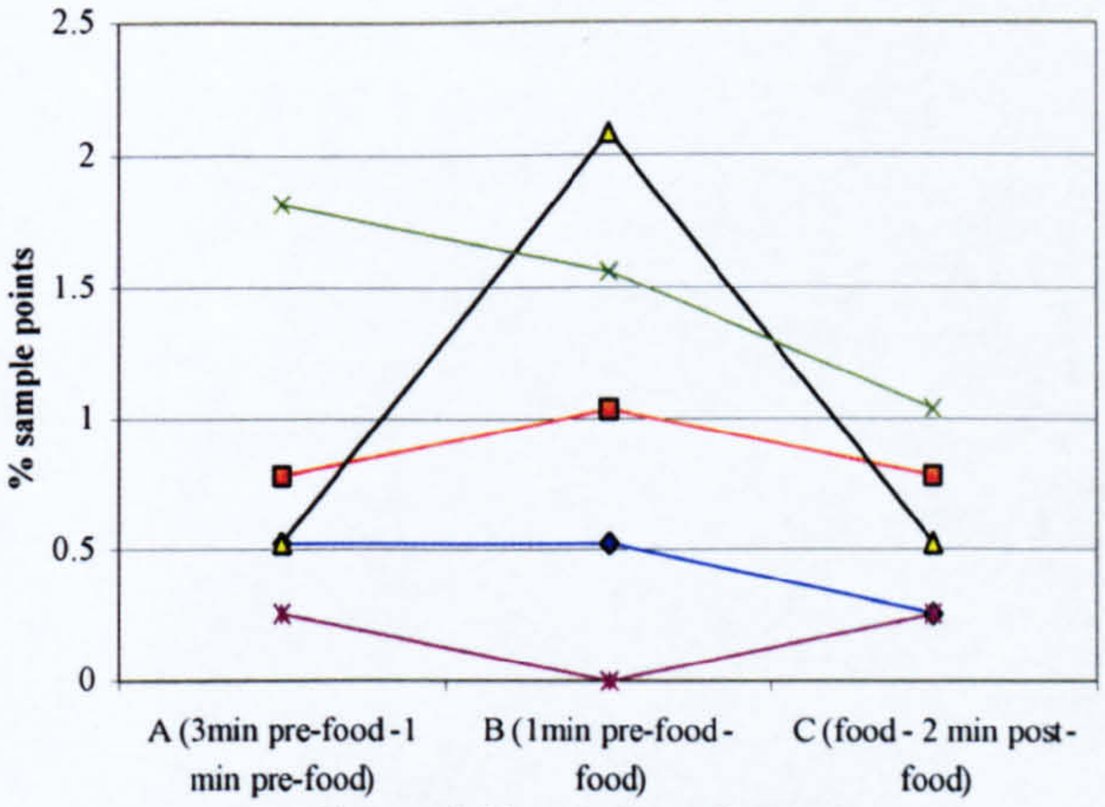
a. Trial period 1 (sessions 1-4)



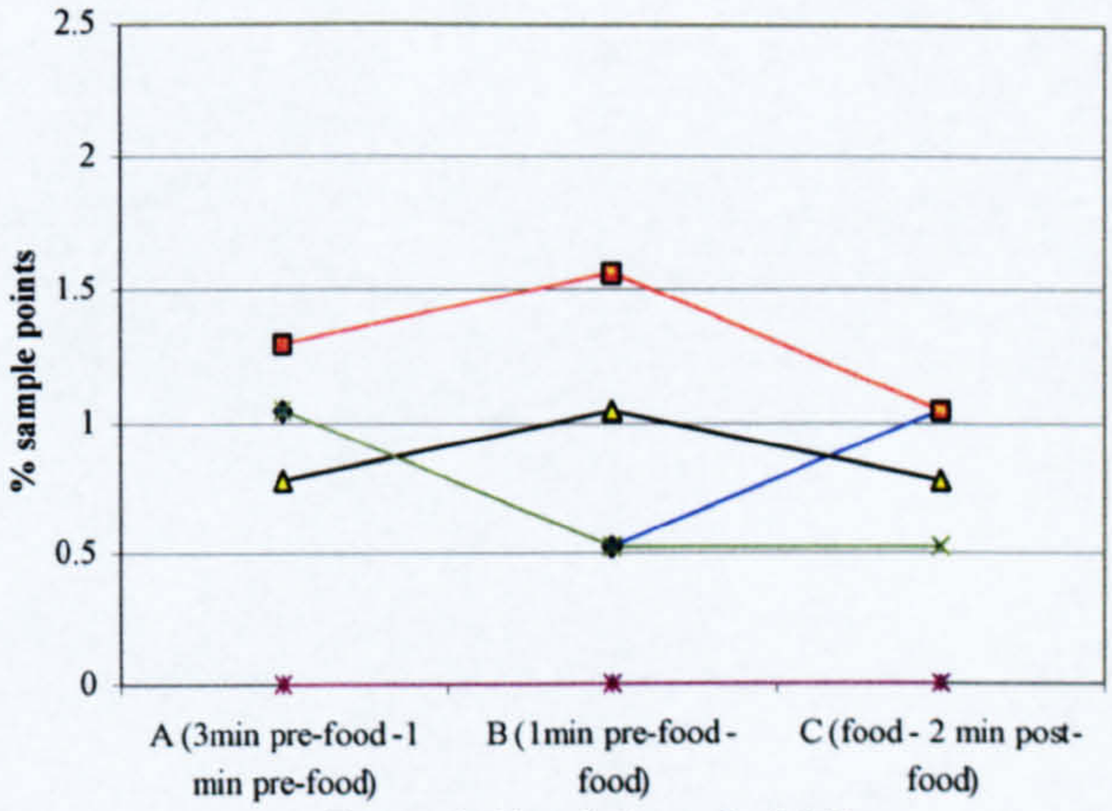
b. Trial period 2 (sessions 5-8)

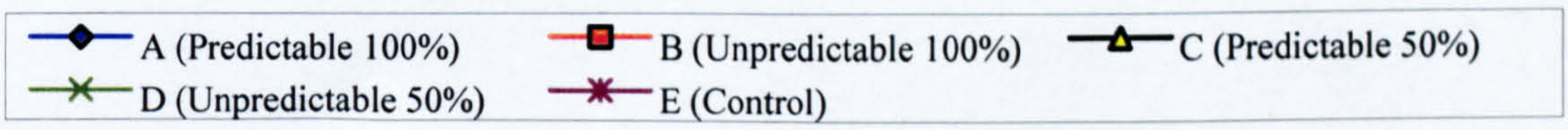


c. Trial period 3 (sessions 9-12)



d. Trial period 4 (sessions 13-16)





DISCUSSION

Inactive (watching observer)

Time spent inactive and watching the observer was unaffected by predictability of feeding and trial period. It was, however, significantly affected by time period of observation, with higher rates seen in Time Periods 1 and 2 (the two time periods before feeding had occurred) than in Time Period 3 (after food had been given). This is likely to be due to a reduction in vigilance after food had been delivered, because the animals had become conditioned to the fact that food would only be delivered once during each session.

There was a significant interaction between predictability of feeding and trial period, although the patterns seen are so complex that it is difficult to draw many conclusions from them. The reduction in inactivity (watching observer) which occurs over Trial Periods 1-3 for the control condition may be explained by habituation to the experimental situation. However, it is difficult to explain why rates should rise again for Trial Period 4, when no changes had been made to the procedure for Control animals, and the observer still did not interact with them in any way. Likewise, it is difficult to interpret the fall in rates of inactivity (watching observer) which are seen at Trial Period 2.

The significant interaction between time period and predictability of feeding shows that rates of the behaviour fell to the lowest levels after feeding for Conditions A and B, to an intermediate level for Condition D and remained constant for Condition C and Control animals. The low rates of inactivity (watching observer) seen after feeding for Conditions A and B are probably due to the fact that they received food on 100% of the initial three trial periods. The amount of time spent watching the observer after feeding is likely to have decreased due to a decrease in vigilance, as mentioned above. The intermediate level seen for Condition D animals fits this pattern, as food was delivered on only 50% of these trial periods, so behaviour would have been affected in this way on only half of occasions. However, it is difficult to explain why Condition C animals, which also received food on 50% of occasions, were not affected similarly to Condition D, and instead showed rates similar to Control animals, which showed a constant level of the behaviour over all three Time Periods.

The significant interaction between trial period and time period for this behaviour shows that rates of inactivity (watching observer) were similar for all three time periods for Trial Periods 1 and 4, but lower after food had been delivered (time Period 3) for Trial Periods 2 and 3. The similarity in rates for all three time periods seen in Trial Period 1 may be due to the fact that in the initial trial period, animals may not yet have learned that only food would only be given once per session, so continued to watch the observer after the food delivery. At Trial Periods 2 and 3, animals had learned that it was pointless to continue to watch the observer after food delivery, as no more would be given. In Trial Period 4, no food was delivered, so animals continued to be vigilant and watch the observer throughout the session.

Inactive (not watching observer)

There was no effect of predictability of feeding on rates of inactivity (not watching observer); however, both trial period and time period of observation had an effect. This behaviour is likely to be affected by two separate but interacting factors. Firstly, it is one of the two categories of inactivity, which appear to

decrease with increased stress, as was shown in Chapter 4. Secondly, we might expect time spent watching the observer to be affected by vigilance. Habituation to an observer who does not interact with the animals and is therefore of no interest to them, might be associated with an increase in this behaviour. There was significantly more time spent inactive but not watching the observer in the second trial period than in the first trial period. This is likely to be due to habituation to the experimental situation and the observer, with an associated decrease in both stress-related behaviours and vigilance.

The significant interaction between trial period and predictability of feeding showed that, although there was little difference between the five conditions for Time Period 1 and 2, at Time Periods 3 and 4, the highest rates of the behaviour were seen for the Control condition. This is likely to be due to the fact that this was the only condition in which the observer was not interacting with the animals in any way, so vigilance decreased over the sessions as habituation occurred. Animals in Conditions C and D showed the lowest rates of this behaviour. This may have been due to the fact that they received food on only 50% of the first three trial periods. This may have made them more vigilant and so reduced the amount of time they spent inactive but not watching the observer.

There was a significant effect of time period of observation, with more inactivity (not watching the observer) occurring in Time Period 3 (after the food was given) than Time Period 1 (between three and one minutes before food was given). This was likely to be due to the fact that after the food was given, the animals no longer spent as much time watching the observer, as they would have learned over the sessions that only one piece of food was given per trial, so no more food would be delivered. However, no significant interaction was found between time period of observation and predictability of food delivery, which might have been expected if this was the case. For example, we might have expected a greater increase at Time Period 3 for Conditions A and B, which received food on all sessions in Trial Periods 1-3 and no increase for Control animals, which never received food. Condition C and D animals, which received food on 50% of sessions in Trial Periods 1-3 might have been expected to show an intermediate increase in this behaviour at Time Period 3. Habituation to the experimental situation may have been occurring for all conditions over each session, rather than over the whole trial period. This might account for the fact that this increase between Time Periods 1 and 3 occurred for all conditions equally.

The significant interaction between trial period and time period of observation indicates that rates of inactivity (not watching the observer) were similar for all three time periods for Trial Periods 1, 3 and 4. However, for Trial Period 2, rates were highest for Trial Period 3, intermediate for Time Period 2 and lowest for Time Period 1. It is unclear why this should be the case for this trial period only.

Vocalising

Vocalising was not affected by predictability of feeding *per se*, but this variable did have an effect when in interaction with others. Trial period, however, did have a significant main effect on vocalising, with less vocalising in Trial Period 2 than in either Trial Periods 3 or 4. This is in contrast to the previous study (Chapter 5), where differences in feeding predictability were not associated with changes in vocalisation rates. It is difficult to interpret changes in rates of

vocalisations in relation to stress and welfare in this species, as no differences were found in the amount of vocalising in response to a stressor in the study described in Chapter 4. However, increased frequency of certain types of vocalisations has been shown to occur in various stressful situations in this species (Epple, 1968). Although no differentiation was made between different types of vocalisations was made in the present study, for reasons discussed in Chapter 4, it is possible that the observed increases in vocalisation may indicate an greater stress levels due to heightened arousal as the routines became more established.

The significant interaction between trial period and predictability of feeding shows a similar pattern for Conditions A, C, D and the Control condition as was seen for scent marking and scratching. This suggests that all three of these behaviours were affected in similar ways by the two variables. It may therefore be the case that vocalisation is affected by stress in a similar way to scratching and scent marking, that is, increased stress levels result in higher rates of vocalising. There are several possible reasons why this relationship was not found in the study described in Chapter 4. Vocalising may be more easily affected by extraneous variables, such as the behaviour of other animals in the colony room, or movements of laboratory technicians, than behaviours such as scratching and scent marking. This could result in 'noise' in the data that would mask any more subtle experimental effects. Increased vocalisation may be a less sensitive measure of stress than either scratching or scent marking, and was therefore not affected by the relatively mild stressor employed in the earlier study. If this were the case, it would mean that the stress resulting from variations in predictability of feeding was fairly severe, at least in comparison to handling and removal of animals from their home cage and cagemate. This is despite the fact that the food whose predictability was manipulated was only a titbit, rather than the main meal of the day. This might have important implications for the results of this study, as it would mean that variations in predictability of feeding, and conceivably other husbandry events, have the potential to cause severe stress to captive animals.

Rates of vocalising for Condition A animals increased throughout the study period. This pattern differed from those seen for the same condition for scratching and scent marking, as for these behaviours rates remained constant, or decreased slightly as the study progressed. The reason for this is unclear.

The interaction between predictability of feeding and time period indicated that rates of vocalisation were constant, and low, throughout all three time periods, for Control animals. Rates were also constant throughout the three time periods for Condition D (the most unpredictable condition), but were higher than those seen for the Control condition. If vocalising is accepted as an indicator of stress it appears, from these results, that a highly unpredictable feeding schedule may result in generally raised levels of stress around feeding time. The two moderately predictable conditions (Conditions B and C) both showed increased vocalisation in the second time period (one minute before food was delivered). This suggests that for animals fed on a moderately unpredictable schedule, stress levels increase in the period around feeding time, but are reduced when food has been delivered. The reasons for the pattern seen for Condition A animals, for which rates were lowest at Time Period 2, are unclear, but may be better understood with reference to the significant three-way interaction between all the variables.

The significant interaction between time period and trial period indicates that patterns over the four Trial periods are similar for Time Periods 1 and 3. Here, rates for Trial Periods 1 and 2 are similar, but lower than those for Trial Periods 3

and 4, which are also similar. The reason for the increase between Trial Periods 2 and 3 is unclear. The pattern for Time Period 2 was slightly different, as there was a decrease between Trial Periods 1 and 2, and a sharp increase between periods 2 and 3, with another decrease at period 4. It is possible that any effects on vocalisation seen as the study progressed, due to factors such as habituation, establishment of the routine or the change in the routine when food was withheld, might be more obvious in this period as it is directly before food delivery. Feeding is likely to be the most important point in the experimental period, and behavioural observations collected nearest in time to this point therefore might be expected to be most affected by feeding and the feeding schedule.

The three-way interaction between the variables shows a fairly constant, low rate of vocalising throughout all stages of the study for the Control animals. This is as would be expected if vocalising was an indicator of stress. Rates for animals exposed to a highly predictable feeding routine (Condition A) were similar to those for Control animals, except for Trial Period 4, where they increased. This is a similar pattern to those seen for scratching and scent marking, both of which are thought to be reliable indicators of stress in this species (see Chapter 4). However, the deviation from Control values occurred at all time periods in the final trial period, instead of only at Time Period 3 (after feeding would have occurred) as was seen in the three-way interaction for scent marking. If vocalising is indeed an indicator of stress in the common marmoset, this would indicate that there is a general increase in stress around the whole feeding period when food is withheld from animals that had previously received it on a highly predictable schedule. However, it should be borne in mind that there is no evidence that increased vocalisation, based on data collected with no differentiation between types of vocalisation, does represent an increase in stress (see Chapter 4). Therefore conclusions based on these data should be viewed with caution.